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Quantification of pralidoxime methylsulfate (Contrathion®) in human urine by capillary zone electrophoresis

Pascal Houzé [a,c,*], Hafedh Thabet [b], Alexandre Delfour [a], Lucile Larrouy [a], Thierry Le Bricon [a], Frédéric J. Baud [c]

[a] *Département De Pharamcologie, Laboratoire De Biochimie A, Hôpital Saint Louis (AP-HP), 1 Avenue Claude Vellefaux, 75010 Paris, France*
[b] *Réanimation Médicale, Centre d'Assistance Médicale Urgente, 10 rue Aboulkacem Chebbi, 1008 Monfleury, Tunis, Tunisie*
[c] *INSERM U 705, UMR 71-57, Université Paris V, Université Paris VII, Hôpital F. Widal, 200 rue du Faubourg St Denis, 75010 Paris, France*

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Abstract

Pralidoxime methylsulfate (Contrathion®) is widely used to treat organophosphate poisoning. For the first time, we developed a specific assay for urinary pralidoxime using capillary zone electrophoresis (CZE) in the following conditions: fused-silica capillary (length: 47 cm, internal diameter: 75 μm), electrolyte solution: 25 mM sodium borate (pH 9.1), voltage: 15 kV, temperature: 25 °C, injection time: 1 or 2 s, on-line UV detection: 280 nm. Sample preparation did not require a deproteinization step (1:5 dilution in water). The method was linear between 0.125 and 2 mg mL^{-1} of pralidoxime (quantification limit: 0.10 mg mL^{-1}). Coefficients of variation for intra- and inter-assay precision were below 10% for all three control levels (0.15–1.15 mg mL^{-1}). This assay was successfully applied to urine specimens from organophosphate poisoned patients treated by Contrathion® ($n = 10$). This CZE method allows the measure of pralidoxime in urine within 15 min with excellent precision, selectivity, and sensitivity. It is simple (no pretreatment) and convenient, thus suitable for the monitoring of Contrathion® therapy in organophosphate poisoned patients.

Keywords: Pralidoxime; Capillary zone electrophoresis; Urine

1. Introduction

According to the World Health Organization, organophosphates are annually involved in about 3,000,000 of poisoning cases with about 10% of mortality [1]. Organophosphate poisonings result mostly from accidental causes and suicidal attempts, especially in developing countries. Standardized therapeutic scheme includes supportive treatment, antidote infusion (atropine and/or oximes) and decontamination [2]. Oximes are nucleophilic agents able to re-activate phosphorylated receptors by binding to organophosphorus compounds [3]. Among approved oximes [4,5], pralidoxime is used as a methylsulfate (Contrathion®) (Fig. 1a) or chloride salts (Protopam®) and obidoxime as dichloride salts (Toxogonine®) (Fig. 1b). Oxime pharmacokinetic is characterized by a rapid plasma decrease secondary to renal excretion [4]. Non-renal elimination only accounts for 5% of pralidoxime total clearance (versus 15% for obidoxime) [6,7]. Supportive treatments, including artificial ventilation and cardiovascular support, might significantly modify oxime pharmacokinetic [8]. It would be, therefore, particularly helpful to obtain additional information about oxime urinary excretion to optimize their administration, especially in severely poisoned patients [8].

Several techniques have been described to measure oxime concentration in plasma [9–11] and in automatic injection device [12]. Two high liquid performance chromatography (HPLC) methods have been published for urine analysis [9,13]. They require 1 mL urine volume and a preliminary deproteinization step. Capillary electrophoresis (CZE) has never been used for oxime determination in urine, although CZE is often a method of choice to determine pharmaceutical compounds [14,15] in preparations and biological samples [16]. We developed a rapid CZE method to measure pralidoxime concentration in a small volume of urine (200 μL) and without sample deproteiniza-

* Corresponding author. Tel.: +33 1 42 49 92 99.
E-mail address: pascal.houze@sls.ap-hop-paris.fr (P. Houzé).

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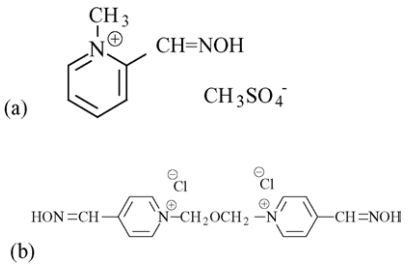

Fig. 1. Chemical structures of pralidoxime and obidoxime. (a) Pralidoxime methylsulfate (Contrathion®, MW = 137 as free base) and (b) obidoxime chloride (Toxogonine®, MW = 287 as free base).

tion. Daily urinary pralidoxime excretion was determined in ten patients treated with a continuous infusion of Contrathion® for organophosphate poisoning.

2. Experimental

2.1. Reagents

SERB laboratories (Paris, France) kindly provided pralidoxime methylsulfate. Sodium decahydrate tetrahydroborate was obtained from Merck (Nogent-sur-Marne, France). Normal Lyphochek Quantitative Urine Control® was purchased from Bio-Rad laboratories (Marnes-la-Coquette, France). Obidoxime, hypoxanthine, xanthine, cytosine, guanosine, adenosine, 5-fluorouracile, cytarabine, 6-*O*-methylguanine, and 7-methylguanine were obtained from Sigma–Aldrich (Saint-Quentin Fallavier, France). Distilled water, used to prepare reagents and standards, was from Frésénius (France Pharma, Louviers, France).

2.2. Electrophoresis

Capillary zone electrophoresis (CZE) was performed using the P/ACE 5500 system (Beckman, Gagny, France) equipped with a variable wavelength UV detector. A fused-silica capillary tube [total length (*L*), 57 cm; injector-detector length (*l*), 50 cm; internal diameter (I.D.), 75 μm] was selected. The part of the capillary ensuring electrophoretic separation is maintained at a constant temperature by immersion in a coolant circulating in a cartridge with a rectangular detection window (800 μm × 100 μm).

Electrophoretic separation was performed in normal polarity (input: anode, output: cathode), the capillary being thermostated at 25 °C. Samples were hydrodynamically injected for 1 or 2 s under a pressure of 20 psi. The CZE electrolyte solution consisted of a 25 mM sodium borate solution (pH 9.1) filtered through a 0.45 μm membrane (Polylabo, Strasbourg, France). A constant voltage of 15 kV was applied and direct UV detection was obtained at 280 nm. Data were collected and analyzed by the Gold® System (Beckman).

The capillary was conditioned before each series of analysis with 1N sodium hydroxide (10 min), followed by distilled water (10 min) and a final 5 min equilibrium with CZE electrolyte solution. Between each sample, the capillary was washed with 1N sodium hydroxide (2 min), followed by distilled water (2 min) and a final 2 min equilibrium with CZE electrolyte solution. These washings were intended to improve the reproducibility of the electroosmotic flow and, thus, that of migration time.

2.3. Preparation of calibration standards and controls

Pralidoxime methylsulfate stock solution (4 mg mL^{-1} as pralidoxime base) was prepared in distilled water and stored at −80 °C for up to 6 months. Pralidoxime calibration standards (0.125–2 mg mL^{-1}) were obtained by spiking 200 μL of normal free urine (Bio-Rad) by increasing volumes of pralidoxime stock solution (31.5–500 μL); all standards were completed to 1 mL with distilled water. Point 0 consisted of distilled water (800 μL) and free urine (200 μL). Hypoxanthine stock solution (10 mg mL^{-1}) was prepared in distilled water with 10 μL of sodium hydroxide 1 M and stored at −80 °C. In these conditions, the solution is stable for 6 months.

Three urine samples from patients treated by Contrathion® infusion were used as controls for the precision study: low (C1 = 0.15 mg mL^{-1}), medium (C2 = 0.45 mg mL^{-1}) and high levels (C3 = 1.15 mg mL^{-1}); controls were stored at −80 °C.

2.4. Samples preparations

No extraction procedure was necessary to perform the assay. Urinary controls and samples (200 μL) were first diluted in 700 μL of distilled water. A volume of 100 μL of hypoxanthine stock solution was added to urine standard, controls and samples, as internal standard (IS). Calibration standards and controls were hydrodynamically injected in the capillary for 1 or 2 s (depending on their pralidoxime concentration); patient urine samples were injected for 1 s.

2.5. Correlation to reference technique

Results were compared with a reference HPLC method first developed to measure obidoxime in urine [13] and slightly modified for pralidoxime analysis [11]. Pralidoxime concentration was measured by the developed CZE assay and HPLC in 30 human urine samples.

2.6. Clinical application

Pralidoxime methylsulfate concentration was measured in 30 urine samples from 10 patients treated in the ICU of Tunis Hospital for a severe organophosphate poisoning. After a loading dose of 5 mg kg^{-1}, Contrathion® was infused at the dose of 50 mg kg^{-1} using a perfusion (n = 5) or an electric syringe (n = 5). Urine was collected before therapy, then on a daily basis and for 3 consecutive days following the beginning of Contrathion® therapy. Urine samples were stored at −20 °C until assayed. Pralidoxime urinary excretion was expressed as mean area under curve (AUC) ± standard error of the mean (S.E.M.).