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Clinical screening of paraquat in plasma samples using capillary electrophoresis with contactless conductivity detection: Towards rapid diagnosis and therapeutic treatment of acute paraquat poisoning in Vietnam

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ABSTRACT

The employment of a purpose-made capillary electrophoresis (CE) instrument with capacitively coupled contactless conductivity detection (C^4D) as a simple and cost-effective solution for clinical screening of paraquat in plasma samples for early-stage diagnosis of acute herbicide poisoning is reported. Paraquat was determined using an electrolyte composed of 10 mM histidine adjusted to pH 4 with acetic acid. A detection limit of 0.5 mg/L was achieved. Good agreement between results from CE-C^4D and the confirmation method (HPLC-UV) was obtained, with relative errors for the two pairs of data better than 20% for 31 samples taken from paraquat-intoxicated patients. The results were used by medical doctors for identification and prognosis of acute paraquat poisoning cases. The objective of the work is the deployment of the developed approach in rural areas in Vietnam as a low-cost solution to reduce the mortality rate due to accidental or suicidal ingestion of paraquat.

1. Introduction

Quaternary ammonium herbicides, with paraquat (PQ) being the most common substance, have been used extensively in the world for controlling the growth of weeds and grasses in order to achieve high agricultural productivity. However, these substances have been classified as moderately hazardous compounds by the World Health Organization [1–3]. An exposure to paraquat via accidental or suicidal ingestion is extremely toxic to man and frequently fatal, as a result of multi-organ failure and cardiogenic shock [1,2,4,5]. After ingestion, the compound primarily accumulates in lungs, resulting in acute pulmonary distress. At the same time, it has drastic effects on the gastrointestinal tract, kidneys, liver and heart. Death occurs to the majority of patients within days to weeks whereas survivors suffer severe sequelae [1,4,6]. In Vietnam, due to its ubiquitous presence, accidental or suicidal ingestion of PQ occurs frequently. According to findings at the Poison Control Center – Bach Mai hospital (the largest central hospital in Vietnam), this is the most popularly used compound (even the only herbicide used) by people in rural areas to commit suicide, with more than 300 cases reported in 2014 and 391 cases reported in 2015. In most of the cases, the patients, unconscious following acute poisoning, were transported to central hospitals where state-of-the-art equipment is available for clinical diagnosis and treatment. Due to the time lost during the transport and the waiting period at central hospitals, which are always overburdened, the majority (80%) of the patients suffered death. Also according to the Poison Control Center, this mortality rate would have been significantly reduced if acute poisoning by PQ could be identified immediately at a local hospital followed by urgent treatment with blood filtration. This however has not been the case due to the lack of inexpensive and easy to use analytical instruments and methods which can be deployed in decentralized hospitals in Vietnam.

The paraquat concentration in plasma has been used as the most common marker to evaluate the possibility and severity of PQ poisoning. The likelihood of survival is higher for patients whose plasma paraquat levels are less than 2.0, 0.6, 0.3, 0.16, and 0.1 mg/L after 4, 6, 10, 16, and 24 h, respectively [7,8]. Plasma levels higher than 2 mg/L are considered to be fatal in most cases [1,9].

The identification of PQ poisoning, the prognosis and the efficiency

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of the treatment require first an analysis of PQ in plasma. So far, the most cited techniques for analysis of paraquat in biological samples have been LC–MS (see for example [5,10–12]), GC–MS [2,13] and HPLC with ultraviolet detection (UV) [14–18]. These instrumental methods however are not affordable for many (local) hospitals or clinical centers in developing countries. In Vietnam, paraquat in plasma could to date only be quantified at few central hospitals which are equipped with HPLC-UV, such as Bach Mai hospital. There is therefore interest in the development of simpler and less expensive analytical techniques which can be deployed even with modest budget and limited expertise. In this context, capillary electrophoresis (CE) can be seen as a more economic alternative. Indeed, CE has been employed for determination of paraquat in water, beer, mung bean and biological samples, using either UV or MS as the detection mode (see [19–25] and other precedent publications listed therein). Note that bench-top CE instruments were used in all these cases. To the knowledge of the authors, the CE approach can be even more cost-effective and better fitted to non-expert use if it is coupled with capacitively coupled contactless conductivity detection (C^4D) [26–28]. Attractive features of C^4D are high versatility, ease in construction and operation, low power consumption and the possibility of miniaturization (see more details in the recent reviews [29,30] and references therein). More conveniently, C^4D can be used in compact portable CE instruments [31–33] for flexible mobile deployment, both of which can also be built in low cost versions [26–28]. Nevertheless, CE-C^4D methodology has to the best of our knowledge not been developed for analysis of paraquat in plasma samples.

Herein a cost-effective and easy to use CE-C^4D method for the screening determination of paraquat in plasma samples using a purpose-made compact instrument is reported. The method developed was applied at the central hospital (Bach Mai hospital) for diagnosis of paraquat acute-poisoning from PQ-intoxicated patients. The results obtained from 31 plasma samples were crossed checked with the well-established HPLC-UV method. The collected data were used by the doctors for mortality prognosis and prescription of therapeutic treatments to paraquat-intoxicated patients.

2. Experimental

2.1. Chemicals and materials

All chemicals used in this study were of analytical reagent grade. Paraquat in the dichloride salt form purchased from Sigma Aldrich (Hamburg, Germany) was used to prepare stock solutions of paraquat at 200 ppm. The structure of paraquat is shown in Fig. S1 in the electronic supplementary information (ESI). Its pKa value was estimated to be 9–9.5 [34]. L-histidine (His), trichloroacetic acid (TCA), ammonia solution (NH_3 25%), ascorbic acid (Asc), arginine (Arg), methanol, acetic acid (Ace), sodium hydroxide (NaOH), hydrochloric acid (HCl), phosphoric acid (H_3PO_4) 85%, ethylenediaminetetraacetic acid (EDTA), cetyltrimethylammonium bromide (CTAB), sodium 1-heptanesulfonate, potassium chloride (KCl) and polyethylenglycol 400 (PEG 400) were purchased either from Merck or Fluka (Germany). Commercial SPE cartridges (Sep-Pak@Vac 3cc), each containing 500 mg of packing material (octadecyl bonded silica particles of 55 µm diameter), were purchased from Waters (Massachusetts, USA).

Polyimide coated fused silica capillaries of 75 µm I.D. and 365 µm O.D. (from Polymicro, Phoenix, AZ, USA) with total (L_{tot}) and effective (L_{eff}) lengths of 60 cm and 50 cm respectively were used for separations. Deionised water purified using a system from Water Pro (Labconco, Kansas City, MO, USA) was used for the preparation of all solutions and for sample dilution if required. pH values of solutions were controlled with an HI 2215 Hanna Instruments pH meter (Woonsocket, RI, USA).

2.2. Instrumentation

All CE experiments were performed on a purpose-made portable CE instrument which employs siphoning for sample injection. A miniature Spellman unit (UM25*4, 12 V, 200 µA, Pulborough, UK) was used which provides a maximum of 25 kV. The high voltage (HV) end of the capillary was isolated in a safety cage equipped with a microswitch to interrupt the HV when the lid was opened. Detection was carried out with a commercial C^4D (ER815, eDAQ, Australia). As both CE and C^4D require a 12 V DC power supply, either mains power or batteries can be used to power the CE-C^4D system.

For cross-check measurements, an HPLC instrument (model: HPLC 1200, Agilent, USA) coupled with a diode-array detector (DAD) was employed. These chromatographic separations were carried out with a reversed-phase column (Zorbax C8, 4.6 mm ID × 150 mm, 5 µm packing materials) and a guard column (Zorbax, ZC8-10C5). Buffer capacities of different buffer compositions were estimated with the Phoebus program from Analis (Suarlée, Belgium).

2.3. Procedures

The plasma samples from PQ-intoxicated patients were provided by the Poison Control Center (Bach Mai hospital). The procedure for treatment of plasma samples was as follows: 2 mL plasma sample containing PQ after addition of 0.4 mL TCA and 0.6 mL H_2O was centrifuged at 4000 rpm for 10 min. 0.5 mL NH_4OH (25%) and 50 µL EDTA (final concentrations varying from 0 to 10^{-2} M) were then added to the obtained supernatant after centrifugation. The resulting mixture (pH 9) was flushed at 1 mL/min through a C18 cartridge which had been activated with 3 mL CTAB (1.4 mM) prepared in NH_4OH 0.1% and 5 mL sodium 1-heptanesulfonate (100 mM) prepared in NH_4OH 0.1%. The cartridge that retained PQ was washed with 10 mL H_2O and 10 mL methanol at the flowrate of 2.5 mL/min. Elution of PQ was then carried out by passing 4 mL acetic acid 8% in methanol through the cartridge at the flowrate of 0.5 mL/min. The eluent was dried with nitrogen and the residual was dissolved in 1 mL acetic acid 1–8% in methanol. A 2 fold enrichment of PQ was achieved with this SPE procedure. The obtained solution was injected into the separation capillary by siphoning at the height of 10 cm for 30 s. The capillary before use was preconditioned with 1 M NaOH for 10 min and deionised water for 10 min prior to flushing with buffer for at least 2 h. Electrophoretic separations were then carried out under a voltage of + 20 kV and with the optimized BGE composed of His 10 mM adjusted to pH 4 with acetic acid (refer to Section 3.2 for more details on BGE optimization). Detection with C^4D was implemented at an excitation voltage of 500 V, an excitation frequency of 600 kHz, a low-pass filter at 1 Hz and with the gain mode within the measurement range of 1 V. To minimize the temperature-induced fluctuation of baseline signals during C^4D recording, a reference signal was subtracted from the signal of the separation capillary. The reference signal was obtained with a reference capillary which was filled with a solution composed of BGE (90% v/v) and deionized water (10% v/v).

For cross check measurements, the HPLC method developed by the Poison Control Centre was used. The method was adapted from those reported elsewhere [15,35]. Briefly, to 1 mL plasma 1 mL TCA 5% v/v was added and this was then centrifuged at 4000 rpm for 15 min. The transparent supernatant was filtered through cellulose acetate membranes (0.2 µm) prior to injection into the HPLC instrument using a sample loop of 30 µL. Chromatographic separation of PQ was carried out at the isocratic mode at the flowrate of 0.5 mL/min using a mobile phase composed of 5% ACN and 95% buffer. For preparation of the buffer, 1.1 g heptanesulfonate sodium, 2 g KCl, 2 mL PEG 400 and 200 mL methanol were added into a 1000 mL volumetric flask, which was then filled up close to the graduated level with deionized water. The pH of the mobile phase was adjusted to 2.5 with H_3PO_4 before precisely adjusting the level of the solution to the graduated mark with