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Case Report

Hair analysis of an unusual case of Chloroquine intoxication

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ABSTRACT

A dead body of middle aged man was exhumed from 6.5 month earth-grave. Autopsy findings were non-specific as the body was completely putrefied. Deceased's scalp hair and kidney was sent for toxicological analysis. Hair sample (50 mg) was incubated with 1 M NaOH (2 ml). Chloroquine was detected in hair and kidney during basic drug screen performed on GC/MS. For confirmation and quantitation, chloroquine was extracted using Hypersep verify CX SPE cartridges while mass detector was operated in SIM mode using the ions of *m*/*z* 245.0, 290.1, 319.0 for chloroquine while ions of *m*/*z* 260 and 455 were monitored for nalorphine (internal standard). Chloroquine was present in high concentration in hair (211 ng/mg) as well as in kidney (37.3 mg/kg). Moreover, chloroquine was not detected in the wash solvents, suggesting ingestion of the drug rather than an external contamination of hair. These findings strongly suggested the acute exposure of higher doses of chloroquine to the deceased before death.

1. Introduction

Chloroquine is a 4-aminoquinoline drug which has been widely used in prophylaxis and treatment of malaria [1]. It has also been successfully employed in patients with rheumatoid arthritis, systemic lupus erythematosus, discoid lupus erythematosus, porphyrea cutane tarda, antiphospholipid antibody syndrome, polymorphous light eruptions, solar urticaria, recurrent basal cell carcinoma of the skin, and in some rarer conditions [2–5]. It is almost completely absorbed after oral ingestion [6]. Although this compound has 30–70% plasma protein binding [7–12] but it is well distributed throughout the body [13]. After administration of single dose in rats, the order of concentration of chloroquine in various tissues from greatest to least was uvea > liver > lung > kidney > vitreous > heart > skin > hair > brain > blood > serum [14]. The results were similar to distribution pattern in rabbits and humans [2,16]. Chloroquine induced retinopathy is one of most significant side effects of chronic, high-dose chloroquine therapy for rheumatoid arthritis, discoid and systematic lupus erythematosus [17]. It has a strong acute toxicity to the heart if one overdoses. It causes significant reduction in blood pressure and produces conduction disturbances sometimes with fatal arrhythmia [18]. Within half hour of oral ingestion of an overdose, the patients experience nausea, drowsiness, tremor, vomiting and abdominal pain. Furthermore clinical characteristics of toxicity include hypokalemia, cardiac arrhythmia, respiratory depression and coma. In the case of an overdose or an acute intoxication, the drug is relatively toxic owing to its narrow therapeutic index [19]. Fatal chloroquine poisonings in either suicide or murder cases has been described in different body fluids and postmortem specimens in various studies [18–28]. However for the first time, hair analysis results from an unusual case of intoxication with chloroquine are presented here.

2. Case history

A middle-aged man died in his bed room apparently due to heart attack. The deceased was buried by family member in local graveyard. After few months deceased family came to know that his wife had murdered him because she had an extramarital affair. The case was registered in local police station against the woman. During the investigation, the woman admitted that she had been giving some tablets (3–4 tablets mixed in meals) to her husband for last two years. On the day of death she had given several tablets mixed in meal in order to intoxicate him. After 6.5 month burial, the grave was exhumed in compliance with court orders and autopsy was performed. The body was completely putrefied and no specific autopsy findings were reported, therefore toxicological analysis is essential to determine the cause of death. Scalp hairs and partially putrefied kidney were sent for toxicological analysis in author's laboratory.

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3. Materials and methods

3.1. Chemical and reagents

Standard chloroquine (powder) was purchased from Sigma–Aldrich (Steinheim, Germany) and nalorphine (1 mg/ml in methanol) from Cerilliant (Round Rock, TX, USA). All solvents were purchased from Acros Organics (Geel, Belgium). Hypersep verify CX SPE cartridges were purchased from Thermo Scientific (Waltham, MA, USA.) Negative hairs were collected from healthy volunteer who had provided written consent about the experimental use of his hair.

3.2. Sample preparation

Scalp hair samples were washed with deionized water to remove dust and soil particles. These hair specimens were washed twice with dichloromethane for 5 min at room temperature. The wash extracts were transferred into a clean tube, evaporated to dryness with nitrogen in a Turbovap evaporator (Zymark, Hopkinton, MA) at 40 °C, and refrigerated at 4 °C until GC/MS analysis was performed to determine the presence of external contaminations. The cut hairs were subsequently dried, weighed (50 mg), and cut into small pieces. After fortifying with 50 μl of nalorphine (10 mg/l) as internal standard, hair specimens were incubated with 2 ml of 1 M NaOH solution at room temperature for 12 h. Negative hair (50 mg) were also digested in 1 M NaOH solution (2 ml) using same procedure. The pH of hair solution was adjusted to 9 with 6 N HCl before analysis [33].

Kidney tissue (6 g) was homogenized using electronic homogenizer and diluted to 30 mL with deionized water

3.3. Toxicological analysis

3.3.1. Screening tests

Screening tests were performed for drug of abuse (benzodiazepines, opiates and barbiturates), acidic/neutral and basic drugs [34,35].

3.3.2. Procedure for quantitation of chloroquine

A stock solution of chloroquine (1 mg/ml) was prepared from the powder. Chloroquine and nalorphine solutions were individually diluted in methanol to prepare working solutions at 100 and 10 μg/ml. The working solution of chloroquine was spiked appropriately into blank hair solution (1 ml) in order to get calibrators of 5, 50, 100, 300 and 400 ng/mg. All calibrators along with the incubated hair sample (1 ml) of victim were taken into 15 ml plastic tubes. After spiking 50 μl of working solution of internal standard, protein precipitation was carried out using acetonitrile (1 ml). 0.1 M sodium phosphate buffer (3 ml, pH 6) was added into each tube and allowed to stand for 5 min. All tubes were centrifuged for 15 min at 4000 rpm. The specimens were then decanted into the SPE extraction tubes after conditioning with methanol (3 ml), deionized water (2 ml) and 100 mM sodium phosphate buffer (1.5 ml). The columns were rinsed with 2 ml of deionized water, acetate buffer (100 mM, pH 4.5) and methanol. Finally columns were eluated with 3 ml of a freshly prepared elution solvent comprising of methylene chloride and isopropanol (80:20 v/v) with 2% ammonium hydroxide. The organic layer was dried under a stream of nitrogen at 50 °C using the Zymark Turbo Vap. To each tube 25 μl of ethyl acetate and 25 μl of BSTFA (derivatizing reagent) were added. All tubes were capped and placed on heat block at 75 °C for 30 min. After cooling to room temperature samples were taken into GC vials for analysis.

A model 7890-A GC coupled with a 5975 mass selective detector was operated in electron impact mode (EI) and selected ion monitoring (SIM) (Agilent Technologies, Melbourne, Australia). A 30-m DB-5 MS fused-silica column coated with a 5% phenyl methyl silicone liquid phase with 0.25-μm i.d. and 0.25-mm film thickness (Agilent Technologies) was used. High-purity helium was used as the carrier gas. Samples were injected (2 μL) using a model 7693 series auto sampler and G4513-A series injector operating in splitless mode, with temperature zones for the injector port set at 240 °C, quadrupole at 150 °C and ion source at 230 °C. The oven temperature program was set for an initial temperature of 120 °C for 2 min and increased to final temperature of 300 °C at 10 °C/min. The total run time was 20 min. In SIM mode, ions monitored for detection of chloroquine were *m*/*z* 245.0, 290.1, 319.0 while ions of *m*/*z* 260 and 455 were monitored for nalorphine (internal standard).

The concentration of chloroquine was also determined in kidney using previously reported method [19].

3.3.3. Validation study

In order to construct calibration curves, 1 ml of scalp hair was spiked with the studied chloroquine at final concentrations ranging from 5 to 400 ng/mg of hair and was analyzed with the described procedure. The method was validated according to criteria established by the SWG-TOX guidelines for linearity, recovery, limits of detection and quantitation, precision and accuracy [37]. Calibration curve was obtained by plotting the peak area ratio of chloroquine to IS versus the amount of the drug, and linearity range was examined. The limit of detection (LOD) and limit of quantitation (LOQ) was estimated at a signal-to-noise ratio equal to 3 and 10 respectively in spiked hair samples. Intraday and interday precision as relative standard deviation (%) and accuracy as relative error (%) were calculated on the basis of the calibration curve.

4. Results

4.1. Identification of chloroquine

Chloroquine was detected in hair and kidney during basic drug screen performed on GC/MS. It was identified by retention time and comparison of generated mass spectra with library spectra (NIST match greater than 91%) [15]. Fig. 1 shows the total ion current (TIC) chromatogram and mass spectrum of chloroquine in the hair extract obtained by GC/MS. Other screening tests were negative for drug of abuses, acidic and neutral drugs.

4.2. Results of validation study

The method has shown good linearity up to 400 ng/mg (r^2 = 0.998). The limit of detection and quantitation were 2.5 ng/mg and 5 ng/mg respectively. The extraction recovery of the compound was between 82% and 89%. The method has good precision (3.4–9.5% CV) and accuracy (analytical variability ranged from −5% to +7.4%) as shown in Table 1.

4.3. Concentration of chloroquine in the hair and kidney of the deceased

For quantitation the mass detector was operated in SIM mode using the ions *m*/*z* 245.0, 290.1, 319.0. The latter was used for quantitation. Nalorphine ions *m*/*z* 260 and 455 were also monitored. Chloroquine peak was appeared at 18.881 min while nalorphine peak was observed at 18.706 min. Following GC/MS analysis of the hair sample, the peak area of the ion *m*/*z* 319 was determined for each extract. Finally chloroquine was quantitated