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Brief communication

HPLC analysis of anti-malaria agent, chloroquine in blood and tissue from forensic autopsy cases in Tanzania

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Abstract

HPLC analysis of anti-malaria agent, chloroquine (CQ) in blood and tissues with a simple HCl back extraction method was applied to three forensic autopsy cases in Dar es Salaam, Tanzania. CQ concentrations in femoral vein blood were 8.5, 48.4 and 43.8 μg/ml in three cases, respectively, which were high enough to attribute the cause of deaths to an acute CQ poisoning. There were great site dependent variations in blood CQ levels. The right heart blood samples were very high, which may be explained by incomplete distribution of the drug before death or postmortem diffusion from liver and its surrounding blood, as high CQ levels were remarkable in the liver. Suicidal and accidental CQ poisonings are very common and CQ is a very important chemical in the field of forensic toxicology in Tanzania.

Keywords: Chloroquine; HPLC; Tissue distribution; Acute poisoning; Tanzania

1. Introduction

Malaria is still the most important parasitic disease in the tropical areas. According to the WHO report, mortality due to malaria in the world is estimated to be over one million deaths each year [1]. Chloroquine (CQ) is a 4-aminoquinolein derivative that was first synthesized in 1934 and has been the drug most commonly used for the treatment and prevention of all forms of malaria [2]. Unfortunately, increasing numbers of *Plasmodium falciparum* strains have become resistant to CQ [3]. Nevertheless, CQ remains the first choice drug for the treatment of malaria in some developing countries, because the drug is inexpensive and has fewer side effects in therapeutic doses compared to other anti-malaria drugs such as fansidar and proguanil.

We have been doing an international joint research work on neuropathology of HIV-1 infection [4] with the Department of Histopathology and Morbid Anatomy, School of Medicine, Muhimbili University

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College of Health Sciences in Tanzania. During the study, we have experienced forensic autopsies of acute CQ poisoning cases, which occur rarely in Japan and other developed countries. CQ-induced retinopathy is well known as one of side effects of chronic, high-dose therapy of CQ for rheumatoid arthritis and discoid and systematic lupus erythematosus [5]. On the other hand, CQ has a strong acute toxicity to the heart if one overdoses. It causes hypotension and conduction disturbances sometimes with fatal arrhythmia [6]. Suicidal and accidental deaths due to acute CQ poisoning are very common in Tanzania.

We present HPLC analysis of CQ in blood and tissues in three acute poisoning cases in Tanzania, Africa. Occurrences of suicidal and accidental CQ poisonings in Tanzania are also introduced.

2. Materials and methods

2.1. Autopsy cases

Table 1 shows three acute CQ poisoning cases, which were autopsied in the Department of Histopathology and Morbid Anatomy, Muhimbili University College of Health Sciences, Faculty of Medicine, Dar es Salaam, Tanzania in 2 months from July to August 2000. All three cases were suicide and police investigation strongly suggested CQ ingestion. No screening tests for the drug were performed at autopsy. No remarkable autopsy findings were seen except for congested lungs with paetechea in Case 2 and congested and edematous lungs with froth in the mouth and nostrils in Case 3.

Table 1
Forensic autopsy cases of chloroquine poisoning in Dar es Salaam, Tanzania

	Sex	Age	Autopsy findings
Case 1	Male	34	Fatty, no marks of external violence
Case 2	Female	21	No marks of external violence, congested lungs with petechea, gravid uterus about 16 weeks
Case 3	Male	24	Well nourished, no marks of external violence, froth in the mouth and nostrils, congested and edematous lungs

2.2. Chemicals

Chloroquine diphosphate was obtained from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals were of analytical grade, and purchased through local suppliers.

2.3. Sample collection

Blood and tissue samples were collected at autopsy. Femoral vein (FV) blood samples were obtained by using a plastic catheter inserted into the femoral veins. Left and right heart blood (LH blood and RH blood) samples were taken by a direct needle puncture of the heart. All samples were stored at −20 °C until analysis.

2.4. Extraction procedures

Blood samples were diluted with an appropriate volume of distilled water, if necessary. Solid tissue samples (1 g) were homogenized with 9 ml distilled water. One milliliter of sample was pipetted into a 10 ml screw-capped glass tube containing 4 ml ether and 0.4 μg of strychnine nitrate salt as an internal standard (I.S.). The mixture was then basified with 1 ml of 2 M NaOH and whirl-mixed for 1 min. The organic phase was clarified by centrifugation for 10 min at 2000 rpm. Then, the organic layer was transferred into another tube and rotated with 0.4 ml of 0.1 M HCl for 15 min and centrifuged. Twenty microliters of the HCl layer were injected into the HPLC system. Standard samples were prepared by adding known amounts of CQ (0–100 μg) as well as 0.4 μg of I.S. to blank whole blood.

2.5. HPLC conditions

HPLC was performed on a TOSOH SC-8020 (TOSHO Co., Tokyo, Japan) equipped with an UV 8020 detector, an AS-8020 autosampler and an online degasser. The HPLC column used was TSK gel Super-ODS column (100×4.6 mm I.D., TOSOH Co., Tokyo, Japan). The mobile phase was acetonitril/20 mM 1-heptanesulfonic acid containing 0.07% diethylamine adjusted to pH 3.4 with phosphoric acid (30:70, v/v). The UV detector was monitored at

254 nm. The column temperature was 40 °C and flow rate was 1.0 ml/min.

3. Results

Fig. 1 shows a HPLC chromatogram of LH blood sample in Case 1. The retention time of CQ corresponded to that obtained from standard CQ samples. Detection limit of CQ by the present method was 0.05 μg/ml in blood samples. Excellent linearity was obtained in response to CQ over the concentration range of 0.05–10 μg/ml. The correlation coefficient values were between 0.98 and 0.99 in all runs. Extraction recoveries of spiked CQ (5 μg/ml) in blood were 79 ± 5% ($n = 4$). There were some small minor peaks of metabolites of CQ (mono-desethyl CQ and bi-desethyl CQ) on the chromatogram of blood and tissue samples. Quantitative analysis of those metabolites was not performed.

Table 2 shows analytical results of CQ in blood and tissues in the three poisoning cases. The concentrations of CQ in FV blood were 8.5, 48.4 and 43.8 μg/ml in Cases 1–3, respectively. RH blood showed remarkable high concentrations compared to those of LH and FV blood samples. In tissue samples, CQ levels were high in the liver and the kidney.

4. Discussion

We introduced the HCl back extraction method [7] to the HPLC determination of CQ in both blood and tissue samples and obtained satisfying results.

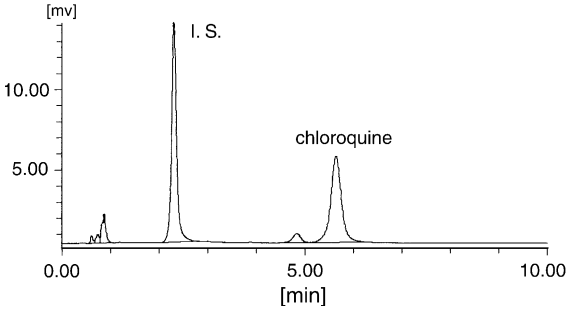

Fig. 1. HPLC chromatogram of chloroquine in a blood sample.

Table 2
Chloroquine concentrations in blood and tissues

Sample	Case 1	Case 2	Case 3
FV blood	8.5 (1.0)	48.4 (1.0)	43.8 (1.0)
LH blood	15.7 (1.9)	42.4 (0.9)	49.9 (1.1)
RH blood	21.8 (2.6)	74.6 (1.5)	168.2(3.8)
Brain	–	–	39.2 (0.9)
Heart	38.4 (4.5)	73.7 (1.5)	115.0 (2.6)
Lung	76.2 (9.0)	76.4 (1.6)	145.1 (3.3)
Liver	–	192.2 (4.0)	584.7 (13.4)
Kidney	211.3 (24.9)	215.9 (4.5)	577.8 (13.2)
Spleen	–	174.4 (3.6)	225.1 (5.2)

FV, femoral vein; LH, left heart; RH, right heart, μg/ml or g; ( ): ratio to FV blood; –, no specimen available.

Various analytical methods have been applied to determine CQ in biological specimen. These include GC [2], HPLC [8] and GC-MS [9]. Houze et al. [10] reported a HPLC method with a simple one step ether extraction procedure for blood and urine samples. Also two-step extraction procedures including acidic back-extraction have been reported to the HPLC analysis of CQ in blood, urine and dried blood on filter paper [11]. The present HPLC method reporting here can be used not only for blood and urine but also for tissue samples with a fine chromatogram.

There were great site dependent variations in blood CQ levels. The RH blood samples were very high compared to LH and FV blood. Incomplete distribution of the drug during life or postmortem diffusion of the drug from the liver and its surrounding blood [12] may explain this phenomenon, as high tissue levels were remarkable in the liver. It was reported that symptoms appeared in times ranging from a few minutes to 4 h after CQ ingestion and the interval between CQ ingestion and death was from 1 to 11 h [2]. Also high CQ levels in the portal vein and the hepatic vein can be expected after a large oral dose of the drug.

Blood CQ concentrations of 3–16 μg/ml were reported in fatal cases [13], and CQ levels in blood in the present cases were high enough to attribute the cause of deaths to an acute CQ poisoning. People receiving a daily 250 mg oral dose for at least 2 months had serum concentrations of 0.2–0.4 μg/ml [14]. In comparison, levels in our cases are significantly higher. Also, high tissue levels were remarkable in the liver and the kidney, which strongly

Table 3
Chloroquine analysis in Tanzanian Government Chemist Agency

Year	Number of analysis		
	Viscera[a]	Stomach contents	Blood
1998	81	57	2
1999	42	22	0
2000	85	36	4

[a] Kidney and/or liver.

suggested acute fatal overdose of CQ. It is suggested that a liver concentration exceeding 150 μg/g is a more reliable indicator for acute fatal CQ poisonings [15]. As no specific autopsy findings for acute CQ poisonings were reported [2] as in the present cases, toxicological analysis of the drug is essential to determine the cause of death.

Actual numbers of acute CQ poisoning in Tanzania are not known, because there is incomplete death registration that leads to under-reporting. However, Tanzanian forensic pathologists recognize well that CQ is the most common drug for suicide in Tanzania. Typically the victim swallows up to 20 tablets of 250 mg CQ each. In an acute overdose, CQ produces respiratory depression and circulatory shock. Typical fatal cases are seen in young pregnant women who overdose the drug for the purpose of abortion, in young females with various mental stresses and also in suicide cases of HIV positive people. According to the data from The Tanzanian Government Chemist Laboratory Agency, total 125 CQ analysis were performed by using a simple UV method in the year 2000 [16] (Table 3). Therefore, fatal CQ poisoning cases could be more than 100 per year in the Dar es Salaam area, whose population is about three million.

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