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Suicide by self-injection of chlormequat trademark C5SUN[®]

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Abstract

Chlormequat is a quaternary ammonium used as plant growth regulating agent. We report here the first suicide case involving a 45 year-old farmer man who intentionally self-injected C5SUN[®], containing chlormequat and choline. An original liquid chromatography high resolution mass spectrometry method (LC-HR-MS), using an hybrid quadrupole-orbitrap mass spectrometer, was developed for qualitative and quantitative analysis of chlormequat in different biological matrices. Toxicological analyses of post-mortem samples highlighted the presence of chlormequat in the blood (2.25 mg/L) and the urine (4.45 mg/L), in addition to ethanol impregnation blood (1.15 g/L). The route of administration (subcutaneous injection) was confirmed by the detection of chlormequat in the abdominal fat sample (chlormequat : 10.04 mg/g) taken from the traumatic injury location, as well as in the syringe found at the death scene, close to the victim's body. Based on the results of these postmortem investigations, the cause of death was determined to be consecutive to cardiac dysrhythmia and cardiac arrest following chlormequat self-injection.

Keywords: suicide, poisoning, chlormequat, CCC, C5SUN[®].

1. Introduction

Chlormequat (2-chloro-N,N,N-trimethylethanaminium) is a plant growth regulator. It is a quaternary ammonium compound, widely used to promote flower formation, to improve fruit setting in fruits and vegetables and reduce vegetative growth and the inhibition of sprouting [1-3]. Chlormequat is commonly applied in the form of the salt chlormequat-chloride or chlorocholine chloride (CCC). CCC is known to be a competitive inhibitor of cholinesterase in animals [4]. This anticholinesterase chemical induces a cholinergic syndrome throughout the central and peripheral nervous system [4]. Administration of anticholinesterase agents may cause cholinergic crisis manifested by symptoms such as sweating, increased salivation, visual disturbance, pulmonary edema, bradycardia, cardiac dysrhythmias, seizures, and eventually ventricular fibrillation and cardiac arrest [5]. Neurological disorders may include confusion and coma [5].

An illegal use of CCC has been reported especially in the case of animals' euthanasia [6,7], and the ingestion of CCC as a method of suicide is circulating through Internet social networks. Until now, at least 10 fatal cases of CCC poisoning have been published [6, 8-10], and all of these cases were reputed from ingestion of the plant growth regulator (CCC). A main limitation of all the published cases is the lack of analytical and forensic results, especially the quantification of the target substance in the different biological samples.

In the present report, an original case of a suicide after subcutaneous self-injection of CCC is presented for a 45 year-old man, with complete autopsy findings and toxicological results. Toxicological analysis of the different post-mortem samples is carried out after development of a specific and original liquid chromatography high resolution mass spectrometry (LC-HR-MS) method for the identification and quantification of CCC in biological matrices. Therefore, the outlines of the analytical method are described and the measurement of CCC concentrations is used for determining the cause of death.

2. Case report

2.1 Case history

A 45 year-old Caucasian farmer man was found dead at home, lying on his bed in a supine position. The victim was covered, fully clothed and a belt loosened. The deceased's clothes were intact and did not reveal any evidence of struggle or violence, no bleeding wounds were observed at any site. A 20ml Terumo[®] Syringe with a 1.2 x 40 mm needle and a plastic 10L

canister of C5SUN® were found in the immediate vicinity of the body. Emergency services attempted performing cardiopulmonary resuscitation without success. The victim had no specific medical history and did not undertake any treatment. The macroscopic external examination of the body at the death scene essentially revealed a 10 x 5 cm bruise of the right iliac fossa with a circa 1 mm diameter injection site (Figure 1).

2.2 Autopsy findings

Autopsy was performed 17 hours post mortem. No lesions were found in the external examination of the body except the bruise with injection site previously described. Autopsy showed congestion of internal organs and pulmonary edema. No organs were injured, and although the cause and manner of death were not established, a traumatic cause was definitely excluded. No pathological previous state was noted. Femoral blood, urine and abdominal subcutaneous fat underlying the injection site were sampled for toxicological analysis. The heart was taken for histopathological analysis, which and showed no macroscopic or microscopic abnormalities. 100 mL of the solution contained in the C5SUN® canister and the syringe were also sent for toxicological analysis.

3. Toxicological analysis

3.1. Volatile compounds analysis

Ethanol as well as other volatile compounds (acetone, isopropanol, and methanol) was determined by gas chromatography-flame ionisation detection-headspace (GC-FID-HS) in the C5SUN® canister liquid contents and blood sample.

3.2. Chlormequat analysis

Both qualitative and quantitative analyses of chlormequat were performed using liquid chromatography high resolution mass spectrometry detection (LC-HR-MS), equipped with a Q Exactive™ Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Scientific™, San Jose, USA). Data were acquired in both full-scan and targeted MS² (t-MS²) modes. Chromatographic and spectrometric conditions, as well as retention times and t-MS² parameters of chlormequat, choline and acetylcholine (I.S) are reported respectively in Tables 1 and 2. C5SUN® canister liquid and of syringe contents have been diluted in methanol before injection into LC-HR-MS. For biological sample purification, blood (200 µL) and urine (200 µL) samples were added in 300 µL of 0.1M zinc sulphate solution and supplemented with 500 µL of methanol containing the internal standard (0.2 mg/L). The solution was mixed on vortex for 2.0 min, kept for 10 min at 4.0 °C, and then centrifuged for 10 min at 3000 g.

Supernatants were evaporated to dryness at 50 °C under a stream of nitrogen. Residues were dissolved in 200 µL of LCMS grade water, vortexed and again centrifuged for 5 min at 3000 g, before transferring supernatants into chromatographic vials for LC-HR-MS analysis. Calibration curves were obtained by fortifying chlormequat-free human biological fluids (blood and urine) with working solution of CCC at final concentrations of 0, 0.5, 1.0, 1.5, 2.0 and 2.5 ng/mL. Fat sample (1 g) was diluted in 5 mL of hexane, and then finely homogenized for 10 min with a Polytron PT-1200 homogenizer (Kinematica AG). The resulting solution was then mixed on vortex for 2.0 min, sonicated for 2 h, and again homogenized by turning for 1 hour. For purification, 200 µL of this liquid solution were added in 300 µL of 0.1M zinc sulphate solution and supplemented with 500 µL of methanol containing the internal standard (0.2 mg/L). The solution was mixed on vortex for 2.0 min, kept for 10 min at 4.0 °C, homogenized by turning for 2 hours and then centrifuged for 10 min at 3000 g. The aqueous layer was removed and evaporated to dryness at 50°C under a stream of nitrogen. Residues were dissolved in 200 µL of LC-MS grade water, vortexed and again centrifuged for 5 min at 3000 g, before transferring supernatants into chromatographic vials for LC-HR-MS analysis. Calibration curve was obtained by fortifying chlormequat-free human abdominal fat (provided by the anatomy lab of the Faculty of Medicine, Rennes) with a working solution of CCC at final concentrations of 0, 3, 6, 9, 12 and 15 µg/g.

4. Results

Ethanol was detected in blood sample at a concentration of 1.15 g/L. The liquid solution taken from the plastic 10L canister of C5SUN[®] did not contain ethanol.

LC-HR-MS analysis has shown the presence of chlormequat and choline in all the analyzed samples (liquid solution, syringe contents, blood, urine and abdominal fat samples). Chlormequat was found at a concentration of 2.25 mg/L in the blood, 4.45 mg/L in urine and 10.04 mg/g in the abdominal fat sample (Figure 2). Quantification of choline was not considered necessary to the current context, as explained in the discussion.

5. Discussion

The plant growth regulating agent chlormequat chloride is available in the market under various trade names. The suspected product C5SUN[®] is sold in canisters of various capacities and the chemical composition consists of chlormequat chloride (460 g/L) and choline chloride (120 g/L).

Choline is a dietary component, found in foods as free choline. Animal studies with choline chloride show a low acute toxicity after oral uptake of 5000 mg/kg by weight. Choline has also been reported as the only metabolite of chlormequat [11], however no acute toxicity attributable to choline was observed in humans following oral doses of 3000 mg choline magnesium/day [12]. According to reported low toxicity of choline, it was considered unnecessary the quantification of this substance in our different post-mortem samples.

Chlormequat chloride is barely metabolised and excreted essentially unchanged; choline chloride was the only metabolite identified (up to 3% in urine) [11]. Chlormequat has already been detected and quantified in various matrices such as food, soil and water by using of gas or liquid chromatography coupled with mass spectrometry [13-19]. In biological samples, chlormequat has been quantified in urine and blood by using thin layer chromatography [7], which is not the most suitable technique for biological samples analysis. Chlormequat has also been detected and quantified by the use of liquid chromatography triple quadrupole mass spectrometry in urine [20] and in blood using an external calibration procedure [21]. To our knowledge, this is the first report where chlormequat is identified and quantified in blood, urine and fat samples using liquid chromatography high resolution mass spectrometry (LC-HR-MS). We developed a specific and fast LC-HR-MS method (3 min/run), which was optimized for different biological samples. A quaternary ammonium compound “acetylcholine” was used as internal standard, and a simple methanolic precipitation was applied for different biological samples purification.

Fatal poisonings by chlormequat ingestion have already been reported using various commercial products [6, 8-10]. In all these cases, chlormequat has accidentally or deliberately been ingested and no toxicological results were reported. However, in three of these cases investigations have shown that the victims died after ingesting quantities ranging from 120 mL to 150 mL of chlormequat commercial solutions containing 345 g/L of chlormequat chloride, which corresponded to ingestion of quantities ranging from 40 g to 50 g of

chlormequat chloride. Interestingly, our case describes for the first time another way of chlormequat self-poisoning, which consists in subcutaneous injection. This statement can be explained as follows: a syringe with a needle was found close to the body, and the size of the needle (1.2 x 40 mm) was in good agreement with the traumatic injury observed at the victim's abdominal wall. The bruise can be explained by the blunt force of the injection of 20 mL of solution in the subcutaneous tissues, and by the use of a 1.2 mm diameter needle, whereas subcutaneous injections are usually performed with 0.6 mm diameter needles (maximum). Moreover, the syringe content was identified as chlormequat and choline, which corresponded to the composition of a commercial product C5SUN®, found beside the body. Finally, a confirmation of route of administration was achieved after identification of chlormequat and choline directly in the abdominal fat sampled opposite to the injection site and the bruise. Investigations have shown that the victim was a pig breeder who used this phytosanitary product to euthanize pigs. Although illegal, it seems to be a known practice in the agricultural areas of northern France and Belgium [7]. Therefore, it can be deduced that the farmer has used the same procedure on himself by subcutaneous injecting a lethal dose of a 460 g/L chlormequat hydrochloride using a 20 mL syringe. The injected dose can be estimated in several CCC grams (9.2 g for 20 mL of injected C5SUN®).

The toxicity of chlormequat has been studied in animals [22, 23], and the Low Lethal Dose (LD_{LO}) was estimated at 1 mg/kg and at 10 mg/kg in intravenous and oral route, respectively. For humans, lethal dose hasn't been estimated, but the supposed injected concentration in our case should be toxic. The concentration of chlormequat in the blood was found to be 2.25 mg/L, and 4.45 mg/L in urine. Other fatal poisoning studies did not quantify chlormequat in biological matrix. However, a recent case of non-fatal suicide attempt due to chlormequat intoxication by ingestion of C5SUN® reported the concentrations of chlormequat in the blood of 5.1 mg/L at T₀ and of 0.82 mg/L at 12 hours after the ingestion [21]. In that case, the authors described that the patient was rescued and saved after first aids provided by the patient's father present during the incident, and who performed an epigastric and thoracic cardiac massage causing emesis. Later, the patient was treated with an antibiotic agent, and was also perfused by 500 mL of physiological serum to increase the diuresis. In our case, the death is obviously due to a subcutaneous injection of C5SUN®, with regard to the the presence of chlormequat in the blood, in the urine and in the abdominal fat.

6. Conclusion

We report for the first time a suicide case by self-subcutaneous injection of chlormequat. Toxicological concentrations of this substance were measured using a specific and fast LC-HR-MS method and were reported in blood, urine and abdominal fat, which can document further investigations involving chlormequat in intoxication cases.

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Table 1. Liquid chromatography and mass spectrometry conditions

Liquid chromatography	Mass spectrometry
<ul style="list-style-type: none"> • Column: C18 (15 mm X 2.1, 2.6 μm) • Temperature: 20°C • Mobile phase: <ul style="list-style-type: none"> A: 60 % CH₃COONH₄ (10 mM) B: 40% ACN with 0.1% (v/v) formic acid • Mode: isocratic • Run time: 3 min • Flow rate: 0.3 mL/min • Tray temperature: 15°C • Injected volume: 5 μL 	<ul style="list-style-type: none"> • Source: HESI-II • Probe: 300°C • Mode: positive • Spray voltage: 3 kV • Sheath gas and auxiliary gas: N₂ • Capillary temperature: 300 °C • Source lens: 60 V • Acquisition data: Full scan HR + Targeted MS² • Resolution: 70,000 FWHM • C-trap capacity: 10⁶ charges • Maximum injection time: 100 ms • Mass range: 50 to 150 m/z

Table 2. Multiple reaction monitoring parameters for chlormequat, choline and acetylcholine

Compounds	Chemical formula	RT (min)	Precursor ion [M+H]⁺ (m/z)	NCE (%)	Product ion (m/z)
Chlormequat	C ₅ H ₁₃ ClN	1.57	122.0733	70	58.0659
Choline	C ₅ H ₁₄ NO	1.42	104.1773	70	60.0815
Acetylcholine (I.S)	C ₇ H ₁₆ N ₂	1.52	146.1253	35	87.0446

Figure 1. Bruise and injection site of the abdominal wall



Figure 2. LC-HR-MS data from the abdominal fat sample

