جامعة نايف العربية للعلوم الأمنية
Naif Arab University for Security Sciences

Naif Arab University for Security Sciences
Arab Journal of Forensic Sciences & Forensic Medicine
المجلة العربية لعلوم الأدلة الجنائية والطب الشرعي
https://journals.nauss.edu.sa/index.php/AJFSFM

الجمعية العربية لعلوم الأدلة الجنائية والطب الشرعي
Arab Society for Forensic Sciences and Forensic Medicine

Postmortem Tissue Distribution of Citalopram in a Case of Carbon Monoxide Poisoning

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توزيع عقار السيتالوبرام في الأنسجة بعد الوفاة في حالة التسمم بأول أكسيد الكربون

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Received 30 Jun. 2019; Accepted 10 Dec. 2019; Online 15 Jun. 2020.

Abstract

Citalopram abuse may impair judgment and increase the risk of suicidal thoughts. This case report aims to study the postmortem tissue distribution of citalopram in a case of carbon monoxide poisoning.

Initial analysis was done by both immunoassay and non-targeted GC-MS screening methods. Carbon monoxide was identified and quantified by measuring the carboxyhemoglobin levels in spleen secretion (black bile) and spleen blood using a UV-visible spectrophotometer, while citalopram was identified and quantified by using an LC-MS-MS system.

Initial analysis showed that citalopram was present in all samples determined by immunoassay. The results of carboxyhemoglobin analysis were 85% in the spleen secretion and spleen blood, which are generally fatal levels. The results of LC-MS-MS showed that citalopram concentrations were 0.58 mg/L, 0.37 mg/L, 0.29 mg/L, 0.13 mg/L, 0.10 mg/L, and 0.01 mg/L, in the spleen blood, brain, spleen, kidney, liver and stomach, respectively. The highest concentrations of citalopram, 0.58 mg/L and 0.37 mg/L, were detected in spleen blood and brain tissue, respectively, which could be used as an alternative specimen to blood.

Keywords: *Forensic Science, Citalopram, Carbon Monoxide, Spleen, Postmortem, Distribuation.*

المستخلص

قد يضعف استخدام عقار سيتالوبرام قدرة الحكم على الأمور ويزيد من خطر الأفكار الانتحارية. يهدف هذا التقرير إلى دراسة توزيع مادة السيتالوبرام في الأنسجة ما بعد الوفاة في حالة التسمم بأول أكسيد الكربون.

تم إجراء التحليل الأولي بواسطة الفحوصات المناعية وتقنية GC-MS غير الهادفة. وتم تحديد وقياس أول أكسيد الكربون عن طريق قياس مستويات الكربوكسي هيموجلوبين في إفراز الطحال ودم الطحال باستخدام مقياس الطيف الأشعة فوق البنفسجية، في حين تم تحديد السيتالوبرام وقياسه الكمي باستخدام تقنية LC-MS/MS. أظهرت نتائج التحليل الأولي أن عقار السيتالوبرام موجود في جميع العينات التي تم فحصها بالاختبارات المناعية. وكانت نتائج تحليل الكربوكسي هيموجلوبين 85 % في إفراز الطحال ودم الطحال، والتي هي عادة ما تكون مستويات قاتلة. أظهرت نتائج LC–MS/MS أن تركيزات السيتالوبرام كانت 0.58, 0.37, 0.29, 0.13, 0.10 و 0.01 مغ/ليتر في دم الطحال، الدماغ والطحال والكلى والكبد والمعدة على التوالي. وتم اكتشاف أعلى نسبة تركيز من السيتالوبرام وهي 0.58 مغ/لتر و 0.37 مغ/لتر في دم الطحال وأنسجة المخ، على التوالي، والتي يمكن استخدامها كعينة بديلة للدم.

الكلمات المفتاحية: علوم الأدلة الجنائية، السيتالوبرام، أول أكسيد الكربون، الطحال، ما بعد الوفاة.

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doi: 10.26735/UCDT2862

1. Introduction

Citalopram is a selective serotonin (5-HT) reuptake inhibitor, which is indicated for the treatment of depression. It may impair thinking and judgment and increase the risk of suicidal thoughts [1, 2]. Appropriate monitoring of suicidal risk and close observation of patients who are initiated with citalopram should be considered, especially in the initial few months of therapy or with dose changes [3, 4]. Postmortem analysis of drug concentrations in human biological specimens is important to distinguish whether the drug overdose is a direct cause of death or not [5].

Carbon monoxide is an odorless and colorless gas which is produced when there is incomplete combustion of any carbon containing substance. The toxicity of carbon monoxide is due to greater binding of carbon monoxide to hemoglobin than of oxygen. Therefore, determination of carboxyhemoglobin levels in the blood can estimate the severity of carbon monoxide poisoning [6]. At 50% to 80% of carboxyhemoglobin in blood, the estimated effects on an individual are weak pulse, respiratory depression, and coma followed by death due to respiratory arrest [7]. These effects are mediated through impairing mitochondrial ATP synthesis via inhibition of oxygen delivery to the electron transport chain that leads to a block of the oxygen binding site of hemoglobin [8] and impaired respiratory function of hemoglobin [9].

Toxicological analysis can provide strong evidence concerning cause of death. For example, the presence of high levels of carbon monoxide may indicate that the deceased died as a result of fire, and the presence of psychoactive drugs in fire victims can be taken as evidence of mental impairment or diseases. Carbon monoxide is found to be a cause of death in many fire victims, while alcohol and drugs have rarely been found in these fatalities in fire victims [10]. It is well known that psychoactive drugs have an effect on judgment and physiological capability to survive during toxicological exposure and to react to any other dangerous stimuli [11]. Therefore, determination of the level of psychoactive drugs in fire victims will provide valuable evidence showing if the cause of death is due to carbon monoxide poisoning alone or along with the indirect effects of these drugs.

In this case report, we represent a 35-year-old female who was found dead with severe burns among burnt trees. The investigation revealed that she was suffering from psychiatric disease and was being treated with citalopram. The cause of death was reported as carbon monoxide poisoning. Because of severe blood loss, the specimens submitted to the toxicology center were spleen blood and spleen, in addition to brain, liver, kidney, and stomach. Initial analysis represented that citalopram was found in all the above organs of the victim. This case report aims to study the postmortem tissue distribution of citalopram in a case of carbon monoxide poisoning.

2. Materials and Methods

2.1. Samples Preparation

Five grams of each tissue sample (stomach, liver, kidney, brain, and spleen) were homogenized with 10 ml of deionized water using a stomacher, a quick homogenizer which provides small tissue processing solutions for toxicological analysis.

For immunoassay and GC-MS (non-targeted) analysis, aliquots of stomach, liver, kidney, brain, spleen issue homogenate and spleen blood were screened for drugs of abuse and other drugs, including citalopram, using Randox Evidence and GC-MS, respectively.

Extraction procedure for GC-MS (non-targeted) analysis: samples were extracted by solid phase extraction (SPE) and analyzed by GC-MS as described in the literature (12). To confirm the presence of citalopram, samples were extracted using solid phase extraction applying the method described by United Chemical Company (UCT, Philadelphia, USA) for extraction of antidepressants [13]. Prior to LC-MS analysis, all extracts including calibrators and control samples were completely evaporated under a stream of nitrogen gas and reconstituted with 75 μL of mobile phase.

The carbon monoxide was identified and quantified by measuring the carboxyhemoglobin levels in spleen secretion and spleen blood using a UV-visible spectrophotometer, while citalopram was identified and quantified using an LC-MS-MS system.

2.2. Instrumental analysis (GC-MS; LC-MS-MS)

For GC-MS analysis, all samples were analysed using a single quadruple Agilent Technologies GC-MS instrument, model number 5977B. Two μL of each sample was injected using a fully automated liquid sampler (ALS) into the injection port at 260ºC in splitless mode, and analysis was done according to the previously referred method [12].

For LC-MS-MS analysis, control and calibration samples were prepared from 1 mg/ml citalopram standard (Lipomed, 1 mg free base / 1 ml methanol) spiked in negative kidney homogenate to eliminate matrix effect. Calibration levels were 100, 500, 1000, and 2000 ng/mlL(Figure-1).

Citalopram was identified and quantified using a

LCQ fleet ion trap mass spectrometer (MS-MS) (Thermo Scientific) equipped with a Surveyor LC pump and autosampler. Instruments were linked and controlled by Thermo Xcalibur® software. Liquid chromatography of compounds was carried out on Hyprersil Gold C18 column (150 × 3 mm I.D; particle size, 5 µm by Thermo Scientific) at ambient temperature. Mobile phase consistsed of 0.1% formic acid in acetonitrile (A) and 10M ammonium formate buffer with 0.1% formic acid (B). Mobile phase was delivered in gradient mode as shown in Table-1. MS detector parameters were optimized by directly injecting the standard to the MSD and an autotuning was performed for citalopram and the tune file was saved to be used in the acquisition method. Citalopram was positively charged at LC-MS interface using electro-spray ionization (ESI) and detected by LC-MS-MS in full scan mode for m/z range 150 – 330. Collision induced dissociation (CID) with energy of 27 was applied on precursor m/z 325 to produce qualifier ions of m/z 280 and 307 and quantifier ion of m/z 262.

3. Results and Discussion

The percentage of carboxyhemoglobin was 85% in both spleen blood and spleen squeeze, which are generally fatal levels (Table-2). The best sample for carbon monoxide determination in fire victims are blood from internal vessels and heart, which are less contaminated with atmospheric carbon monoxide at the fire site [14]. However, spleen blood and spleen squeeze can be used as alternative samples when there is severe blood loss [15].

Table 1- *Mobile phase (0.1% formic acid in acetonitrile (A) and 10M ammonium formate buffer with 0.1% formic acid (B)) delivered in gradient mode.*

Step. No	Time (min.)	A %	B %	Flow (µl/min.)
0	0	20	80	240
1	6	90	10	240
2	10	90	10	240
3	11	20	80	240
4	13	20	80	240

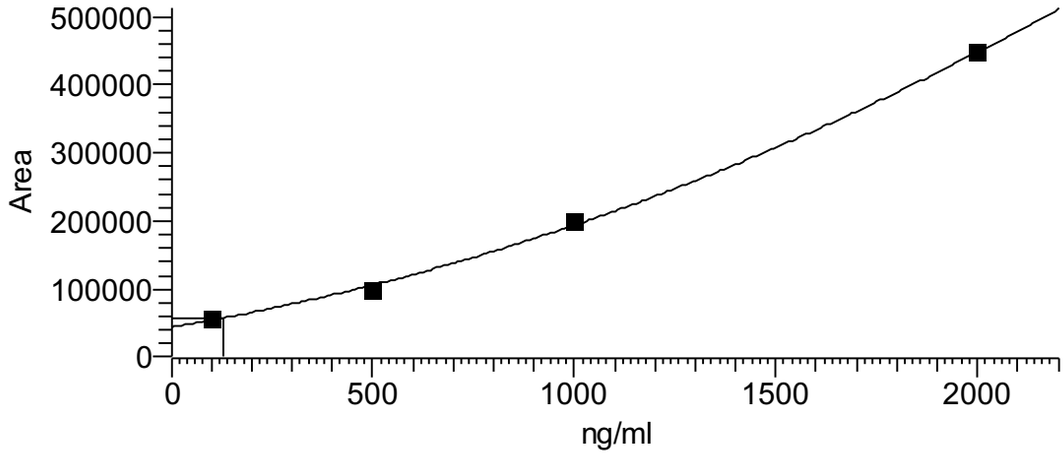

Figure 1- *Calibration curve of citalopram (100, 500, 1000 and 2000 ng/ml) by LCMS-MS. Quadratic regression (R2)= 0.9990.*

Since citalopram was not detected in non-target GC-MS method and there is no specific test for citalopram immunoassay analysis, citalopram was present in all samples. This is due to cross reactivity of citalopram hydrobromide salt with escitalopram metabolite assay in immunoassay analysis by 40.3% and 52.6% for whole blood and urine, respectively, which are applicable in all other matrices according to test kit package insert from Randox laboratories. Therefore, the presence of citalopram was confirmed and measured by LC-MS-MS.

Citalopram levels were 0.58 mg/L, 0.37 mg/L, 0.29 mg/L, 0.13 mg/L, 0.10 mg/L, and 0.01 mg/L, in the spleen blood, brain, spleen, kidney, liver and stomach, respectively (Table-3). The concentrations of citalopram were highest in spleen blood followed by brain tissue. Therefore, spleen blood may be used as an alternative sample in cases of burn victims when other biological fluid samples are unavailable. The levels of citalopram in this case report are within the non-toxic levels, according to previous postmortem studies. Lethal levels of citalopram were previously reported in blood (3.2–49 mg/L), liver (12–55 mg/kg), kidney (13 mg/kg) and brain (2–22 mg/kg) (16).

In this case report, the high level of carbon monoxide revealed that the victim died from inhalation of fire fumes; toxicity has been reported with carbon monoxide levels of 29% to 75% in the spleen [16]. A high level of carbon monoxide has been previously reported in postmortem cases when there are depressant drugs such as alcohol and barbiturates [17]. King [17] noted that the fatal level of carbon monoxide is higher among those who had the highest blood levels of ethanol, which is due to the effect of ethanol on the central nervous system functions. For example, impaired judgment and thinking, that reduces the escape ability. Similarly, we suggest that the high levels of carbon monoxide in this case report may be due the central nervous system effects of citalopram.

Table 2- *Carbon Monoxide Quantitative Result*

Sample	Carbon monoxide Conc.
Spleen	85%
Spleen Blood	85%

Table 3- *Citalopram Quantitative Results.*

Sample	Citalopram Conc. (mg/L)
Spleen Blood	0.58 mg/L
Brain	0.37 mg/L
Spleen Secretion	0.29 mg/L
Kidney	0.13 mg/L
Liver	0.10 mg/L
Stomach	0. 01 mg/L

4. Conclusion

The concentrations of citalopram were within non-toxic levels and consistent with the therapeutic range used by the victim. The postmortem concentration of citalopram in brain, spleen and kidney tissues was determined. This case report could provide additional data about postmortem concentrations and redistribution of citalopram in different organ tissues such as spleen blood and brain in a case of carbon monoxide poisoning associated with blood loss. Patients who are on drugs like citalopram may find it difficult to escape a fire and fall victim to carbon monoxide poisoning.

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