CARDIOVASCULAR DRUG AND SUICIDE DEATH: DETERMINATION OF CARVEDILOL, AMLODIPINE, DOXAZOSINE AND DILTIAZEM IN TWO FATAL CASES

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INTRODUCTION

Both suicide and cardiovascular disease are common cause of death. Suicide is a serious public health problem and a lot of medical conditions have been identified as risk factors for suicide death, including cancer, cardiovascular disease and chronic illness, as well as history of psychiatric disorder. Several studies have reported that individuals with cardiovascular disease experience suicidal ideation at rates varying between 8-17%. In this context, self-poisoning is a common method of suicide, but there is only few studies fully characterizing suicide methods in the presence of major physical health condition.

Cardiac medications were identified as lethal at a significantly higher proportion in cardiovascular disease self-poisoning than in people without disease, who died of self-poisoning. Patients with cardiovascular disease have greater access to certain medications with the possibility of being lethal in overdose. Taking into account the fundamental contributory role that cardiovascular drugs can play in causing death, determining the concentration of cardiovascular drugs in post-mortem fluids and tissues is very important for the correct determination of the cause of death. However, cardiovascular drugs are seldom searched during the analysis in post mortem specimens in forensic cases. In fact, even though cardiovascular drugs are widely used in clinical practice, and it is known that they are potentially lethal in over-dosage, there is scarce data on the toxic/lethal concentrations in the literature, and there is very little information in the literature on the toxicological profile in relation to cause of death.

GOALS

The present study describes two fatal suicidal overdoses involving four cardiovascular drugs (Figure 1): carvedilol (CRVD), doxazosin (DOX), amlodipine (AML) (case 1) and diltiazem (DLTZ) (case 2). Analytical data and case circumstances for these suicide deaths are presented, determining the target compounds in different post-mortem specimens (such as peripheral and central blood, urine, brain, liver, gastric contents) by means of a simple, fast and sensitive procedure using liquid chromatography with tandem mass spectrometry (LC-MS/MS).

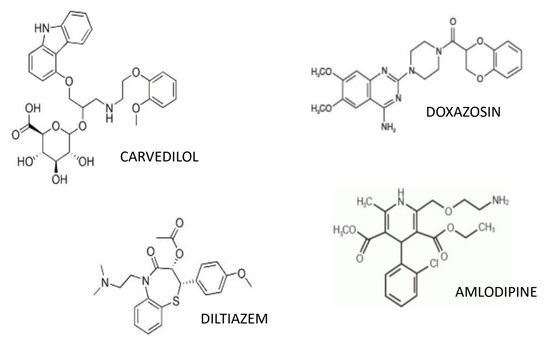

Figure 1: Structure of four cardiovascular drugs

CASE HISTORIES

CASE 1

- 56 year old man, weight 73, height 176.
- He was found unresponsive in his bed by his sister with a suicide note left.
- Several empty bottles of prescription medications were found near the corps: one bottle of DOX (30 tablets per bottle at 2 mg each), a second bottle of DOX (30 tablets per bottle at 4 mg each), one bottle of AML (14 tablets per bottle at 10 mg each) and two bottles of CRVD (30 tablets).
- Anamnestic data were available: epilepsy, heart disease and hypercholesterolemia under pharmacological treatment and a history of depression were reported.
- The corpse was in good condition, well nourished. At the external examination, no traumatic lesions or evidence of violence were observed. At the judicial autopsy, cerebral and pulmonary edema were revealed and all the viscera appeared congested. Subpleural petechiae were observed. Aortocoronary sclerosis was found and the right coronary artery showed a critical stenosis. Partially dissolved tablets were found in the stomach contents (280cc). The histological examination showed sclerosis of the myocardium, in particular in the posterior part of the right wall (Figure 2). Histological investigation of the other organs revealed no other significant findings.

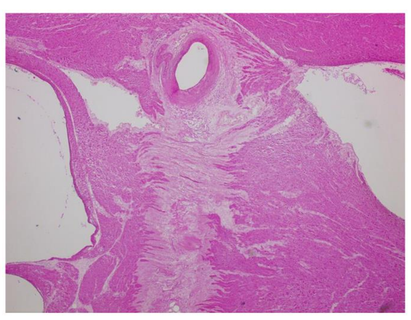

Figure 2: Myocardium, HE, 20X.

CASE 2

- 57 year old woman, weight 84, height 163.
- The woman was found with no signs of life and a condition of rigor and livor mortis was detected
- Eight empty blister units of DILTZ 200 mg, sustained release, were found near the corps.
- Little anamnestic data were available: previous gastroplanty surgery for obesity and a history of hypertensive heart disease, under pharmacological treatment, were reported. She had already attempted suicide in the past.
- The corpse was in good condition, obesity class I, well nourished. At the external examination, no traumatic lesions or evidence of violence were observed. At the autopsy, cerebral and pulmonary edema were revealed and all viscera appeared congested. Subpleural petechiae were observed. Partially dissolved tablets were found in the stomach contents. The histological examination showed sclerosis of the myocardium in the septum fibro-flatty myocardial replacement and focal sings of subendocardial contraction bands (Figure 3). Histological investigation of other organs revealed no other significant findings.

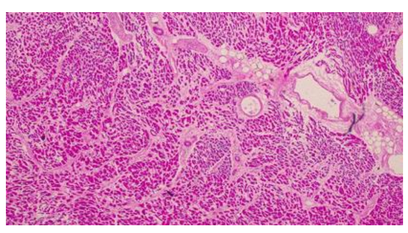

Figure 3: Myocardium, HE, 20X.

MATERIALS and METODS

Biological fluids and tissue sample of the two autopsy cases were taken during autopsy at the University Center of Legal Medicine of Modena and Reggio Emilia, were immediately frozen and were kept at -20°C until analysis.

Peripheral blood was screened for volatiles by headspace gas chromatography with flame ionization detection. A comprehensive screening for pharmaceuticals and drugs of abuse, including NPS and fentanyl analogues (a total of 280 analytes included metabolites), was performed on samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and full scan gas chromatography-mass spectrometry (GC-MS).

As a preliminary result, case 1 screened positive for DOX, AML, CRVD, NH_2-clonazepam (metabolite of clonazepam) and lacosamide; case 2 screened positive for DLTZ, phenobarbital, diazepam and its metabolite nordazepam. Ethanol at concentration of 0.35 g/L was also found in the peripheral blood in case 2. Therefore, we focused our attention on the detection and quantitation of the four cardiovascular drugs: CRVD, DOX, AML and DLTZ. We evaluated the distribution of these analytes in the different collected specimens by the LC–MS/MS method validation for the simultaneous determination of the four analytes in all collected biological samples of the victims. The analytical procedure included the following steps:

SAMPLE PREPARATION
- Body fluids: 200 µL matrix aliquots, 20 µL of IS solution, 1 mL of ammonium formate and 1 mM acetonitrile-methanol solution (70/30) with 0.1% formic acid.
- Tissue samples: 1g of homogenized tissue, 40 µL of IS solution, 2 mL of ammonium formate and 1 mM acetonitrile-methanol solution (70/30) with 0.1% formic acid.
- All samples were mixed and centrifuged for 10' at 3500 x g.

CLEAN-UP
- The total supernatant for body fluids and 1 mL of supernatant for tissues were purified through Phree™ phospholipid removal tubes (1 mL).
- The extract obtained was evaporated using a Concentrator plus Eppendorf.
- All residues were reconstituted with 150 µL of mobile phase solution.

LC-MS/MS ANALYSIS
- High-performance liquid chromatography-tandem mass spectrometry (HJPL-MS/MS) was performed using the Agilent 1200 LC System coupled with a 4000 QTRAP with an electrospray ionization (ESI) Turbo V™ Ion Source.

LC-MS/MS PARAMETERS

Liquid Chromatography
- COLUMN: Synergi 4 µm Polar-RP (150 × 2.0 mm; 4 µm particle size) set to 35°C.
- MOBILE PHASE: (A) ammonium formate 1 mM and 0.1% formic acid in water and (B) ammonium formate 1 mM acetonitrile-methanol solution (70/30) with 0.1% formic acid.
- GRADIENT PROGRAM: 0.0-20.0 min linear gradient from 5% to 95% (B); 20.0-23.0 min isocratic at 95% (B), 23.0-23.2 min linear gradient from 95% to 5% (B)
- FLOW RATE: 0,25 ml/min.
- INJECTED VOLUME: 10 µL .

Mass Spectrometry
- ION-SPRAY VOLTAGE: 5500 V
- SOURCE TEMPERATURE: 450°C
- NEBULIZATION AND HEATING GAS (AIR): 40 psi and 40 psi
- ACQUISITION: Multiple Reaction Monitoring (MRM) (Table 1)
- DWELL TIME: 100 ms

Analyte	MRM transitions (m/z)	DP[$] (Volt)	CE[Y] (v)	R_T [b] (minutes)	IS[P]
CRVD	407.5→224.4[a], 283.1[b], 100.0[b]	60	45, 30, 40	15.81	CRVD-d_5
DLTZ	415.0→178.0[a], 370.0[b]	60	30, 25	14.67	CRVD-d_5
AML	409.3→294.0[a], 238.0[b]	60	18.5, 14	14.99	AML-d_4
DOX	452.3→344.4[a], 247.0[b]	60	43, 30	14.88	PRZS
CRVD-d_5	412.1→104.8[a]	60	28	15.86	-
AML-d_4	413.1 → 238.1[a]	60	15	15.02	-
PRZS[&]	384.0 → 247.0[a]	60	38	14.62	-

Table 1: MRM transitions and LC–MS/MS parameters optimized for the target analytes. CRVD-d_5[£]: CARVEDILOL-d_5; AML-d_4[£]: AMLODIPINE-d_4; PRZS[&]: PRAZOSINE; [P]IS: internal standard; [$]DP: declustering potential; [Y]CE: collision energy; [b]R_T retention time; [a]quantifier transitions are highlighted in bold characters; [b]qualifier transitions are used for confirmation purposes.

RESULTS

The method was validated according to standard practice for method validation in forensic toxicology, ANSI/ASM Standard 036, First Edition 2019 and the calibration model, bias, intra- and inter-day precision, carryover, interferences, matrix effects, limit of quantitation (LOQ)/limit of detection (LOD), recovery, dilution integrity and stability were evaluated. The validation acceptance criteria were met in all parameters. Table 2 gives a summary of the concentrations of the analytes measured in all the matrices and it shows other findings revealed at the routine systematic toxicological analyses. In case 1, the presence of CRVD, DOX and AML was found in all analyzed matrices. The concentration ranges of CRVD, DOX and AML in different specimens were tested at 0.094−1.763 mg/L (mg/kg), 0.109−4.936 mg/L (mg/kg), and 0.869−>20.0 mg/L (mg/kg), respectively. In case 2, the presence of DLTZ was found in all analyzed matrices and the concentration range of DLTZ in all matrices was tested at 16.6-26.5 mg/L (mg/kg).

		PERIPHERAL BLOOD (mg/L)	CENTRAL BLOOD (mg/L)	URINE (mg/L)	GASTRIC CONTENT (mg/L)	BRAIN (mg/Kg)	LIVER (mg/Kg)
Case 1	CRVD	0.500	SA[ζ]	0.094	positive	0.067	1.763
	DOX	0.910	SA[ζ]	0.109	positive	0.435	4.936
	AML	1.278	SA[ζ]	0.869	positive	0.984	>20.000
	OTHER FINDINGS	NH_2-Clonazepam 0.157 Lacosamide 3.820	SA[ζ]	NH_2-Clonazepam 0.062 Lacosamide 4.373	Lacosamide	NH_2-Clonazepam 0.118 Lacosamide 2.315	NH_2-Clonazepam 0.682 Lacosamide 1.427
Case 2	DLTZ	16.600	20.200	SA[ζ]	Positive	16.700	26.500
	OTHER FINDINGS	EtoH 0.35 g/L Phenobarbital 0.870 Diazepam < LLOQ Nordazepam 0.018	Phenobarbital 1.800 Diazepam < LLOQ Nordazepam 0.029	SA[ζ]	/	Phenobarbital Diazepam Nordazepam	Phenobarbital Diazepam Nordazepam

Table 2: CRVD, DLTZ, AML and DOX concentrations in post-mortem specimens and other findings. SA[ζ]: Sample not Available

DISCUSSION

The study led to the implementation of a fast, sensitive and simple method for the detection and quantification of the four commonly prescribed cardiovascular drugs in post-mortem specimens including fluids and tissues for forensic purposes. The concentrations of the target cardiovascular drugs in the different post-mortem specimens are presented, giving information about the lethal data and the distribution of the drugs in the body during fatal intoxication.

As is well known, from a forensic point of view, an accurate collection of circumstantial data, followed by a thorough autopsy, histological investigation and toxicological investigation, is always essential to lead to a correct medical-legal diagnosis and to allow the cause of death to be identified. For both cases under examination, the analysis of the circumstantial data quickly directed the investigations toward the hypothesis that the cause of death had been a drug intoxication with the goal to commit suicide. The pathological findings of the decedents are consistent with death attributable to drug poisoning and in both cases, the histopathological findings show cardiac pathology.

For the presented cases, the toxicological analysis was indispensable to confirm the cause of death, because it gave proof of the presence of elevated concentrations of the cardiovascular drugs in question and enabled a comparison with published post-mortem blood concentrations for fatal cardiovascular intoxication, when available. Case 1 correlates well with reported post-mortem blood concentrations for AML overdose. Instead, no references were available for DOX and CRVD concentrations in fatal cases, but the toxicological findings were noticeably higher than the therapeutic concentrations. Evidence for post-mortem redistribution was unavailable because of the absence of central blood collection. The medical examiner ruled the cause of death as mixed drug intoxication with atherosclerotic coronary vascular disease as a possible contributing factor.

Case 2 presented in our study correlates well with reported post-mortem blood concentrations for DLTZ overdose. The toxicological findings in case 2 demonstrate that the cause of death was determined by acute poisoning of DLTZ alone. No significant evidence for post-mortem redistribution was revealed because of the similarity of the central and peripheral blood concentrations of the drug. The medical examiner ruled the cause of death as DLTZ intoxication, with myocardial fibrosis as a possible contributing factor. For both cases, The manner of death was ruled suicide.

CONCLUSION

✓The post-mortem lethal reference values in two different cases for concentrations of AML, DOX, CRVD and DLTZ in fluids and tissues are presented.
✓These values contribute knowledge to the scarce data reported in the international literature.
✓A careful multidisciplinary evaluation of all data (circumstantial information, autopsy, histological/toxicological investigation) is necessary to assess the contributory role of the substance intake in fatal death.
✓The new data related to DOX and CRVD intoxication concentrations in different post-mortem specimens (both fluids and tissues) are presented.
✓Because many suicide attempts are reported in patients treated with cardiovascular drugs, it is important in all cases of sudden death to perform a toxicological analysis, especially in heart disease patients treated with these drugs, even for widely used and prescribed drugs such as those presented herein.