Flecainide in Cadaveric Blood and Tissues

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Abstract: In this report, we described a death caused by a deliberate overdose of *Flecainide acetate* (Almarytm[®]), an antiarrhythmic agent. The patient had taken a box of 20 Almarytm[®] 100 mg tablets. The Flecainide concentration found in the post-mortem cardiac blood was 10.16 mg/L. This concentration could not have been determined by post-mortem diffusion of the drug from gastric residue because the patient was previously given activated carbon during the emergency procedure. In fact, in the peripheral blood, the Flecainide concentration was 8.64 mg/L, therefore, this concentration is overlapping with the concentration in the cardiac blood; the gastric content was negative at the screening of Flecainide, while the liver tissue concentration of Flecainide was 59.6 mg/L and the bile concentration was 128 mg/L. The brain tissue concentration of Flecainide was 4.19 mg/L. In this case, the cause of death, excluding that toxicity was depending on post-mortem gastric diffusion of the drug, because of the absorbing activity of the administered carbon at the recovery.

Key words: Flecainide, antiarrhythmic, cadaveric blood, gastric content.

1. Introduction

Flecainide acetate (2,5 bis (2,2,2-trifluoroeth-oxy)-N-(2-piperidylmethyl) benzamide acetate MEDA PHARMA) is a sodium channel blocking agent, very effective as antiarrhythmic drug, used in the treatment of ventricular arrhythmias [1]. Flecainide is characterized by good patient tolerance and by a broad spectrum of activity, in fact, it is indicated for the treatment of various arrhythmias including the ventricular arrhythmias [2], as reported also in a recent revue on the management of AF (atrial fibrillation) by Flecainide [3].

Flecainide also has local anaesthetic properties that depress cardiac contractility. After oral administration, Flecainide is rapidly absorbed and peak plasma concentrations occur after 0.5~6 h, with a long plasma half-life of 11 h. Its apparent volume of distribution is high, about 8.7 L/kg, and its plasma protein binding is around 40%. Flecainide undergoes biotransformation via O-dealkylation with the forming of two main metabolites: meta-O-dealkylated Flecainide and meta-O-dealkylated lactam of Flecainide. Both can be

conjugated further [4].

Its plasma pharmacokinetics have been extensively described [5]. The therapeutic levels in serum range between 0.2 mg/L and 1 mg/L. Flecainide is considered toxic at the concentration of 1 mg/L [6]. In toxic doses, hypotension arises rapidly, and the resulting reduction in hepatic and renal blood flow would decrease Flecainide elimination from blood [7]. Post-mortem blood concentration of 13 mg/L and 16.3 mg/L were reported in several studies following ingestion of Flecainide [8, 9]. Overdosing can cause or worsen supraventricular or ventricular arrhythmias and cause heart failure. Overdosage by mouth should be treated by removing the drug from the stomach through lavage, followed by activated charcoal; haemodialysis or haemoperfusion are unlikely to enhance elimination [7-11]. In this case, activated charcoal was administered to the patient.

The present work evaluates the distribution of Flecainide in human tissues and studies the possible exclusion of post-mortem gastric diffusion of this drug caused by the absorbing activity of carbon given during the emergency rescue attempt.

In order to demonstrate this, samples of cardiac blood, peripheral blood, liver, bile, gastric residue and

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brain tissue were analyzed after homogenization.

2. Material and Methods

2.1 Case History

The patient was a 58-year-old woman, suffering of atrial fibrillation, clinically under treatment with Flecainide (Almarytm[®]); recently she changed her dosage, from ¼ tablet of Flecainide two times a day to ½ tablet two time a day.

She committed suicide in December 22, 2009. Regarding circumstances of her death, the husband declared that he had discovered an empty box containing around 20 tablets of Almarytm[®].

Other relevant information comes from reports made available at the hospital: at midnight the patient arrived unconscious and without pulse. Immediately she sent to the ICU (intensive care unit) with a diagnosis of abuse of antidysrhythmic, she had a serious insufficiency cardio circulatory and the medics did a gastrolisis with active carbon 50 mL and dopamine's infusion.

During the night, the atrial pressure was 50/30. At 7:30 a.m., the clinic conditions were terminal; as a matter of fact, there were absence of spontaneous respiration, absence of trunk reflex, pulselessness and hypothermal. She was declared dead around 9:00 in the morning.

Unfortunately, the blood specimens carried out in order to the clinic control are eliminated to the laboratory of the therapy unit. During the autopsy, carried out 24 h after her death, there was no evidence of significant natural disease and no other significant pathological findings.

The heart was morphologically normal; the cardiac arteries presented a light atherosclerotic damage near the origin of the right anterior descending.

The heart was morphologically normal and the coronary arteries were free of atheroma.

Cardiac and femoral blood, gastric residue, liver, bile and brain cortex were sampled for toxicological analysis. Based on the toxicology investigation, the

cause of death was defined.

2.2. Toxicological Analyses

Flecainide acetate was obtained from MEDA PHARMA s.p.a while Nalorfine HBr standard was obtained from S.A.L.A.R.S. (Via San Francesco 5-22100 Como Italy)

A stoke standards solution containing Flecainide acetate was prepared by appropriate dilution in water intravenous use from the mother solution; standards were stored at the temperature of 4 °C.

Other chemicals and solvents were analytical reagent graded. Sylon MSTFA (N-Methyl-N-(trimethyl-sylyl) trifluoroacetamide) was purchased from Sigma-Aldrich (St. Louis, MI, USA).

Blood and tissue samples were collected and frozen at the temperature of -20 °C until analyzed.

Peripheral and cardiac blood, liver, bile, brain and gastric residue samples were added to 4 mL of phosphate buffer pH = 6 and sonicated for 60 min.

Then the samples were centrifugated at 3,000 rpm for 30 min. The pH values of supernatant were measured and fixed to pH 6 through NaOH 1 N.

The samples were extracted using Bond Elute Certify[®] (Varian Palo Alto, CA) cartridges.

The cartridges were activated by pouring twice 2 mL of methanol and 2 mL of phosphate buffer pH = 6.

All samples were spiked with Nalorfine methanol solution, the internal standard, at 0.2 mg/L and then added to the cartridge. The column was washed with 3 mL of Millipore[®] water, 3 mL of HCl 0.1 N and 5 mL of methanol. The samples were eluted by solution of Diclorometane: 2-Propanol: Ammonium hydroxide (20:5:0.5), 2 mL.

Then the elute was evaporated under a gentle stream of nitrogen gas. The residue was resuspended in 0.05 mL of MSTFA (N-Methyl-N-(trimethyl-sylyl) trifluoroacetamide) and derivatized at the temperature of 70 °C for 30 min.

For the screening analysis, an Agilent 6890 gas chromatograph was used in combination with a HP

MSD series 5973 mass spectrometer. The GC conditions were as followed: splitless injection mode (purge time 1 min); HP 5MS 5% phenyl-methyl-silicone capillary column 15 m, 0.25 mm, 0.25 μ m film thickness; Injector port temperature: 250 °C; Oven temperature programmed: 100 °C; (initial time 2.25 min) then 180 °C (rate: 40 °C/min) and then to 290 °C (rate: 10 °C/min); final temperature 310 °C and final run time: 23.08 min; Carrier gas: helium, constant flow-rate 1 mL/min.

For the screening investigation, the MS conditions were as followed: scan mode 50-550 a.m.u threshold: 150 scan/s, ionization energy 70 eV.

Data were automatically processed with the NIST peak search program and with the NIST identification of peak Mass Spectra Library program (Revision 2008) from Agilent Technologies.

For the quantification analysis an Agilent 7890A Gas-Chromatograph was used in combination with HP MSD series 5975 mass spectrometer. The GC conditions were the same used during screening.

The calibration curves were created: one using flecainide-spiked blood at these concentrations: 0, 1, 2, 2.5, 10, 20 mg/L; the second curve using flecainide-spiked brain tissue at the concentrations of: 0, 2, 2.5, 10, 25 mg/L. The third curve using flecainide-spiked liver tissue at these concentrations: 0, 10, 25, 50, 100 mg/L. And finally, the fourth curve has been prepared using flecainide-spiked bile at the concentrations of: 0, 10, 25, 50, 100 mg/L.

The quantifications of Flecainide were performed by SIM MODE: the principal ions of the Flecainide-TMS at 156.00 m/z, 301.10 m/z and 543.00 m/z were selected. The ions of Nalorfine (ISTD) at 313.10 m/z, 324.10 m/z, 414.40 m/z and 440.20 m/z were selected.

The concentrations of Flecainide in the samples were determined from standard curves. A typical chromatogram is shown in Fig. 1.

3. Results and Discussion

The standard curves were linear for Flecainide concentration. Retention times of Flecainide and Nalorfine were, respectively, 7.10 min and 11.09 min. The calibration curves were linear and presented a least-squares regression model ($y = ax + b$).

The correlation coefficient of the calibration curves using Flecainide-spiked blood was 0.974 for the 2~20 mg/L range and the correlation coefficient of the calibration curves using Flecainide-spiked brain was 0.960 for the 2.5~25 mg/L range. The correlation coefficient of the calibration curves using flecainide-spiked liver was 0.957 for the 25~200 mg/L range.

The correlation coefficient of the calibration curves using flecainide-spiked in the bile was 0.982 for the range 25~200 mg/L.

Flecainide concentration in post-mortem cardiac blood was 10.16 mg/L and in peripheral blood was 8.64 mg/L: these two concentrations are overlapping. The brain tissue concentration determined by the GC/MS confirmation technique was 4.19 mg/L. In the liver Flecainide concentration was 59.6 mg/L, while in the bile the concentration was 128 mg/L. The results are presented in Table 1.

Post-mortem changes to drug concentration in blood represent an important factor that should be considered when evaluating its value in forensic toxicology. In this case, Flecainide levels in cardiac and peripheral blood are overlapping; Flecainide levels in blood and tissue agree with analogues evaluations in references.

As for Flecainide, its concentrations are reported to be 3.6-fold higher in post-mortem blood than in ante-mortem blood [10].

Two mechanisms of post-mortem have been reported to determine the increase in concentration of a drug: the passive diffusion from gastric residue [12], and post-mortem redistribution from organs containing

Fig. 1 Characteristic GC/MS spectra of Flecainide-TMS.

Table 1 Flecainide concentrations in fatal cases.

Specimens	Cardiac blood (mg/L)	Peripheral blood (mg/L)	Liver (mg/L)	Bile (mg/L)	Brain tissue (mg/L)	Gastric contents (mg)
Present case	10.16	8.64	59.6	128	4.19	Negative at screening
References (range)	7.3~94	7.3~94	111~550	160~419	33	19~7,000

elevated concentrations of the xenobiotic agent [13]. Many factors can cause an unexpected drug concentration in post-mortem blood: first of all the post-mortem redistribution, represented by the

movement of drug in the body after death, which may result in higher drug concentration in the subject's blood at autopsy than immediately after death. The event that is most likely to influence this phenomenon is the release of the substance from the organ or tissue to the surrounding blood, due to variations in pH levels and to passive diffusion. The latter mechanism is most likely to take place from the stomach content to cardiac and pulmonary blood through the stomach wall [14].

In this case, the possibility of a post-mortem gastric diffusion of the drug appears improbable because of the negative result in the gastric residue by GC/MS technique; in our opinion this fact is due to the absorption activity of activated carbon. The blood concentration is really a consequence of absorbing metabolic activity before reanimation measures.

Nevertheless, redistribution of Flecainide from tissues towards blood vessels is of little importance, without gastric redistribution [9]. The circumstances surrounding the death pointed to a self-poisoning with Flecainide. In fact the toxicological analysis detected that the cardiac blood concentration of Flecainide was far over the range of toxicity; the therapeutic dose of 200 mg corresponds to a plasma concentration of 214~281 µg/L (average 251) in healthy subjects [5], and the concentration of 3 mg/L of Flecainide is considered toxic [15].

So, in our opinion, also taking into account that the toxic amount ingested has caused a slowed down metabolism, on the bases of the reported drug levels the cause of death was due to Flecainide overdose.

Furthermore, drug concentration in post-mortem blood has to be carefully evaluated in reference to liver and encephalic levels, along with information from the autopsy and the circumstances of death.

4. Conclusions

Flecainide acetate is an antiarrhythmic compound. Overdosing can cause or worsen supraventricular or ventricular arrhythmias and heart failure. However,

the interpretation of drugs in post-mortem blood and tissue remains difficult because many drugs are unstable, depending on post-mortem redistribution, and also on the circumstances under which death occurred.

In this suicidal case, the toxicological analysis demonstrated that drug concentration in peripheral blood (far over the range of toxicity), along with tissues concentrations, was the only cause of death, excluding that toxicity was depending on post-mortem gastric diffusion of the drug, because of the absorbing activity of the administered carbon at the recovery.

As in any forensic case of intoxication by drug, the possibility that post-mortem factors (and particularly the redistribution from stomach content to the surrounding fluids) may determine extremely high concentrations of the examined substances has to be taken into account when evaluating the actual cause of death.

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