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Case report

Toxicological investigations, including hair testing, in a death involving gabapentin

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Abstract

Gabapentin, a GABA analogue, is a drug with anticonvulsant properties, used to treat epilepsy in adults and to relieve pain in patients suffering from postherpetic neuralgia. The usual oral daily dose is 300-1800 mg, but may be increased to a maximum of 3600 mg in 1-3 divided doses. The most reported adverse effects of gabapentin are somnolence, dizziness, ataxia, fatigue and impaired vision. The drug is often associated with opiates in the addict population. A 58-year-old man was found dead at his home. He was treated by gabapentin since 3 years for major neuropathic pain and a depressive syndrome. Body examination did not reveal traumatic injury. Following an initial screening of peripheral blood, all the biological specimens collected during autopsy were analysed, using a dedicated liquid chromatography-tandem mass spectrometry method for gabapentin. Analyses revealed intoxication with gabapentin, including cardiac blood concentration at 33800 µg/L, peripheral blood at 42800 µg/L and urine at 854200 µg/L. Hair (2 cm, brown) tested positive at 421 pg/mg. The toxicological significance of the measured concentration was difficult to establish because of the limited identification of gabapentin in human hair. These results appear to be compatible with an overdose of gabapentin and the pathologist attributed the death to a fatal intoxication.

Key words

Gabapentin, forensic toxicology, postmortem concentration, hair, UPLC-MS/MS

Introduction

Gabapentin (1-(aminomethyl)cyclohexaneacetic acid) is an analog of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) that is used for its anticonvulsant properties. It is indicated as adjunctive therapy in the treatment of partial seizures in adults with epilepsy and for pain relief in patient with postherpetic neuralgia (Neurontin®, Horizant®, Gralise®). The drug is available in 100-400 mg capsules, 600-800 mg tablets and in a 250 mg/5mL solution. The usual oral daily dose is 300-1800 mg, but may be increased to a maximum of 3600 mg in 1-3 divided doses [1]. Gabapentin is not appreciably metabolized in humans and is mostly eliminated from the systemic circulation by renal excretion as unchanged drug. Its half-life is 5 to 7 hours, which is unaltered by dose or multiple administrations [2]. At therapeutic dosage, gabapentin can present adverse effects including somnolence, dizziness, ataxia, fatigue and nystagmus [1].

Several intoxications cases due to gabapentin have been reported in the literature, including serious adverse effects and even deaths [3-14]. No range of fatal blood concentration could be established by the various authors. Postmortem blood concentrations were generally higher than 10000 µg/L and the ratio cardiac blood to peripheral blood was not in favor of postmortem redistribution. In France, since 2-3 years, a gabapentinoïd (gabapentin, pregabalin) crisis has been observed which resulted in several pregabalin-related deaths, but none with gabapentin [15]. A comprehensive review about gabapentinoïds has been recently published, summarizing current knowledge an epidemiology, pharmacology and analytical aspects [16].

The objective of this publication is to present the analytical procedure established for the determination of gabapentin in postmortem specimens, as this drug is not detected using standard screening procedures. In addition, according to national statistics [15], this is the first published case of gabapentin-related death.

Case history

A 58-year-old man (56 kg, 1.70 m) was found dead at his home. Limited putrefaction, with a greenish coloring of the superficial venous system over the abdomen, was observed. A recent prescription from a neurologist was found at his home. The prescription included gabapentin (1 tablet of 800 mg and 1 capsule of 100 mg, each morning, noon and evening, i.e. 2.700 mg/day), paracetamol (1000 mg, when needed) and bromazepam (1 tablet of 6 mg in the evening). According to the information from his doctor, the victim was treated since 2017 for severe neuropathic pain, and a depressive syndrome. Gabapentin dosage was increased in 2018 and in mid-2019, due to persistent pain. An external examination of his body was carried out by the medical examiner, but no cause of death was identified. No traumatic injury was noticed. During this examination, peripheral blood, cardiac blood,

urine and hair (2 cm, brown, not oriented) were collected for toxicological investigations. No autopsy was performed.

Toxicological analyses

The blood and urine specimens were submitted to standard postmortem toxicological investigations, including determination of ethanol (HS-GC/FID), a screening for volatiles (HS-GC/MS), several screenings for pharmaceuticals and illegal drugs (ELISA, LC-DAD and LC-MS/MS). A specific MRM method for gabapentin testing in postmortem fluids and hair was developed on a Waters TDQ, as there was indication that the subject was under therapy from his medical records.

Sample preparation was performed by protein precipitation from 100 μ L of fluids (cardiac blood, peripheral blood and urine) in presence of 100 ng of diazepam- d_5 used as internal standard and 2 mL acetonitrile. After agitation and centrifugation, acetonitrile was evaporated to dryness, and the residue was reconstituted in 30 μ L of 5 mM ammonium formate buffer adjusted at pH 3. Hair analysis was performed after decontamination with two baths of 5 mL dichloromethane for 2 min. Then, the hair stand was cut into small pieces (< 1 mm). Gabapentin was extracted from 25 mg decontaminated cut hair in the presence of 25 ng of diazepam- d_5 (25 μ L of a 1 mg/L solution in acetonitrile), after incubation in 1 mL acetonitrile and placement in an ultrasound bath for 2h at room temperature. After agitation and centrifugation, acetonitrile was evaporated to dryness, and the residue was reconstituted in 30 μ L of 5 mM ammonium formate buffer adjusted at pH 3.

Chromatography was achieved using a Waters HSS C18 column (150 x 2.1 mm x 1.8mm) maintained at 50 °C in a thermostatically controlled enclosure. A gradient elution was performed using formate buffer adjusted to pH 3 (mobile phase A) and 0.1 % formic acid in acetonitrile LC-MS grade (mobile phase B), as mobile phases at flow rate of 0.4 mL/min. The initial gradient was 87 % mobile phase A and the final gradient was 5 % mobile phase B, at 7 min. An injection volume of 2 μ L was used in all cases. A Xevo triple quadrupole mass spectrometer was used for the detection of the drugs. Ionization was achieved using electrospray in the positive ionization mode (ES+).

The following conditions were found to be optimal for the analysis of gabapentin and the internal standard: capillary voltage at 1.5 kV, source block temperature at 149 °C, desolvation gas nitrogen heated at 600 °C and delivered at a flow rate of 1000 L/h. In order to establish appropriate multiple reaction monitoring condition, the cone voltage was adjusted to maximize the intensity of the protonated molecular ion, and collision-induced dissociation of both species was performed. Collision energy was adjusted to optimize the signal for two most abundant product ions of gabapentin: m/z 171.9 > 136.8 (CV, 32 V; CE, 16 eV) and 171.9 > 57.8 (CV, 32 V; CE, 24 eV), and the most abundant product ion of diazepam- d_5 : m/z 290.1 > 198.2 (CV, 56 V; CE, 32 eV). Transition m/z

171.9 > 136.8 was used for quantification of gabapentin. MassLynx 4.1 software was used for quantification.

Linearity in blood was observed in blank blood spiked with gabapentin at concentrations ranging from 100 to 100000 µg/L, with a correlation coefficient of 0.995. QC samples (100 and 10000 µg/L), analyzed in duplicate in six independent experimental assays, were used for determination of a coefficient of variation for precision and accuracy. These CVs were lower than 20 %. The limit of detection and the limit of quantification were established at 1 and 100 µg/L, respectively. Under the chromatographic conditions used, there was no interference with the analytes by chemicals or any various matrices (matrix effect < 20 %).

Linearity in hair was observed for gabapentin concentrations ranging from 10 to 1000 pg/mg, with a correlation coefficient of 0.999. QC samples (50 and 200 pg/mg), analyzed in duplicate in six independent experimental assays, were used for determination of a coefficient of variation for precision and accuracy. These CVs were lower than 20 %. The limit of detection and the limit of quantification were established at 1 pg/mg and 10 pg/mg, respectively. Under the used chromatographic conditions, there was no interference with the analytes by chemicals or any extractable endogenous materials present in blood or in hair.

Results and Discussion

Although gabapentin and diazepam-d₅ are chemically very different, the rationale for choosing diazepam-d₅ is its use during the initial screenings of each biological specimen and therefore the possibility of direct confirmation of the extract with a more dedicated method, without any further extraction. There was no difficulty to extract diazepam-d₅ in acetonitrile. This choice was already proposed in a previous publication dealing with pregabalin [17]. Of course, the use of deuterated gabapentin represents the gold choice. The screening of blood, during routine lab testing, revealed the absence of ethanol, volatiles, illegal drugs and new psychoactive substances. In cardiac blood, the screening for pharmaceuticals identified paracetamol, bromazepam and codeine. These drugs were quantified in the peripheral blood at: paracetamol (11500 µg/L), bromazepam (270 µg/L) and codeine (20 µg/L). All the concentrations were considered in the therapeutic range or at the high limit of what is acceptable in therapeutic treatment (for bromazepam). In a therapeutic context, gabapentin can be administered concomitantly with analgesics, benzodiazepines or opioids, when patients have severe pain or are depressed. However, even under therapeutic dosage conditions, potentiation of co-administered substances may be possible, particularly if they are central nervous system acting.

To get more information, all the biological specimens collected during external body examination (cardiac blood, peripheral blood, urine and hair) were analysed, using a dedicated UPLC-MS/MS method for gabapentin, according to the protocol described above. The drug tested positive in all the biological matrices, including the hair specimen (Table 1). The central blood/peripheral blood ratio was 0.79. This value is close to the one (0.86) measured by Cantrell et al [7]. Based upon established drug ratio data [18] and given the information available in the literature [19-20], this value suggests a minimal potential for gabapentin postmortem redistribution. This interpretation is also consistent with the concept that acidic compounds with a low volume of distribution are not subject to post-mortem redistribution. This is the case with gabapentin, for which postmortem redistribution is therefore probably negligible, which explain the small difference of concentration between cardiac blood and peripheral blood [19-20]. It seems that the central to peripheral blood ratio for gabapentin is generally lower than 1, as pointed by Hamm et al [8] with a mean value at 0.90 ± 0.24 .

The cardiac blood (33800 µg/L) and peripheral blood (42800 µg/L) concentrations of gabapentin are in the range of toxic values [1], and within the values reported in the literature for fatal overdoses. Häkkinen et al [3] investigated all medico-legal death cases in Finland in which gabapentin was found in postmortem toxicology during 2010 and 2011. Gabapentin was found in 43 cases. The median blood concentration was 12000 µg/L in the abuser group (in combination with opioids) and 8300 µg/L for deaths in the other cases. Tharp et al [4] reported 104 cases where gabapentin was identified in postmortem blood samples. Overall, in 47.1 % of the fatality cases, gabapentin was directly involved in the death. Most gabapentin fatalities had several other intoxicants present (opioids, over-the-counter medications, antianxiety, and antidepressant medications). In cases in which gabapentin was determined to be the cause of death, the blood concentrations ranged from 1100 to 134000 µg/L. Middleton [6] reported the autopsy findings of a 62-year-old woman with a history of depression, whose death was caused by intentional ingestion of gabapentin. Drug concentration was 88000 µg/L in peripheral blood. Cantrell et al [7] presented a fatality case that appeared to be associated with isolated and acute gabapentin ingestion. A 47-year-old woman was found dead at work with a bottle of gabapentin 600 mg. There were 26 tablets missing in the bottle. As it was observed in our case, gabapentin concentration was higher in peripheral blood (37000 µg/L) when compared to cardiac blood (32000 µg/L). Hamm et al [8] reported gabapentin analyses performed on 30 postmortem cases. Overall, gabapentin concentration ranged from < 1000 µg/L to 82000 µg/L in peripheral blood. Almost 50 % of cases showed elevated gabapentin concentrations, between 30000 and 82000 µg/L. Ramoo et al [9] reported the case of a 67-year-old man who died from acute gabapentin intoxication, with a cardiac blood concentration at 180000 µg/L. The highest postmortem concentration of gabapentin even described in the literature was 434000 µg/L [21]. In

view of these data and concentrations, it seems to be very complicated to define a concentration which means impairment or a fatality threshold, since some hospitalized patients have higher gabapentin concentrations than deceased individuals. There is no correlation between the measured concentration and the type of intoxication, with or without a fatal outcome. In addition, Hill et al. [22] suggested that oxycodone-induced tolerance is mediated by Protein Kinase C and that reversal of tolerance by pregabalin may be a contributory factor in oxycodone overdose deaths.

In order to obtain information on the exposure to gabapentin over time, the hair sample collected during the examination of the body was analyzed. This analysis makes it possible to verify the history of consumption. As explained in a previous publication for pregabalin [17], the use of pure acetonitrile as extraction solvent is not very common since it does not swell the hair and is known to precipitate proteins. However, a high extraction recovery combined to analyte stability was observed under these conditions. It is known that acetonitrile is a poor extraction solvent when compared to methanol or mixtures of solvents, but it was considered as the more suitable approach to test for gabapentin during the early validation process. The authors measured 421 pg/mg in a 2 cm brown hair. Based on an average growth rate at 1 cm/month, this length represents what has been incorporated during the two last months. The toxicological significance of the measured concentration was difficult to establish as limited data are available in the medical literature. Indeed, only 2 laboratories have presented data, although including several subjects. However, daily doses, frequency of consumption are missing data, as well as the minimal detectable dosage in hair. Wang et al [23] identified gabapentin at 10 to 56 pg/mg, in 3 subjects treated daily. Musshoff et al [24], in their comprehensive database identified gabapentin at 10 to 10400 pg/mg in 140 cases. However, there is no controlled study about pharmacokinetic parameters of gabapentin incorporation in hair that has been published. It is therefore not possible to interpret the data in terms of dosage and frequency of use, although the victim was a patient treated daily. Despite administration at high dosage (> 1 g/day), gabapentin seems weakly incorporated in hair. Gabapentin has a basic and an acidic group (pKa 3.68 and 10.7) and exists at blood pH in a zwitterionic state. This can explain the limited incorporation in hair, as it was already observed for pregabalin [17] and confirmed for gabapentin [24].

Although the victim was depressive, one cannot establish the moment when gabapentin started to be misused, as it was not possible to achieve a segmental hair test (too short length and absence of root-tip orientation). Smith et al [25] estimated and described the prevalence and effects of motivations behind and risk factors for gabapentin misuse, abuse and diversion. They reviewed 33 papers, including 23 cases studies and 11 epidemiological reports, from 7 countries, including France.

As a general rule, gabapentin is misused in combination with other psychoactive substances such as alcohol, benzodiazepines and opioids. Concomitant use is particularly important, because gabapentin is often co-prescribed with opioids, and patients suffering from pain often receive prescriptions for benzodiazepines due to anxiety and difficulty in sleeping. Substances abuse populations, especially individuals with a history of or current opioid misuse, appear to be at particular risk for misuse. However, an overdose of gabapentin, in the event of severe pain, can also lead to a fatal end. Surveillance and medical monitoring of patients is essential, because, as described by Finlayson et al [26], it is unknown whether synergistic effects of gabapentin with opioids may contribute to central respiratory depression. The authors concluded that additional studies are needed to establish the exact role of gabapentin in these deaths.

In the view of the circumstances of this case and the toxicological findings, the experienced pathologist listed the death as acute poisoning with gabapentin.

Conclusion

The objectives of the present publication were to present: 1. an original analytical approach, 2. document potential post mortem redistribution, 3. add data on the concentration of gabapentin in hair. According to the literature, it appears that gabapentin is often prescribed to patients in association with other substances, such as opioids, analgesics and benzodiazepines. The case presented in this paper illustrates a death involving gabapentin, where paracetamol, bromazepam and codeine were also identified. The measured post-mortem gabapentin concentration appears to be toxic and responsible of the death, but it seems complicated to define a definitive concentration which means impairment or fatality.

At this time, there are few specific publications in the literature dealing with gabapentin identification in human hair, particularly on the analytical approach. This case completes the available data and demonstrates that even daily used in the gram range, the measured gabapentin concentrations are in the pg/mg range in hair.

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Table

Cardiac blood	Peripheral blood	Urines	Hair (2 cm)
33800 µg/L	42800 µg/L	854200 µg/L	421 pg/mg

Table 1: Results of gabapentin in all biological specimens collected during autopsy