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Amitriptyline poisoning of a baby: How informative can hair analysis be?☆

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ABSTRACT

We reported a case of a 6-month-old baby girl who was hospitalized in the pediatric emergency for central nervous system disorders then coma. Toxicology analysis showed the presence of amitriptyline (AMI) and its metabolite nortriptyline (NOR) in blood and urine of the baby. Additional investigations suggested a shaken baby syndrome. Given the family context, a judge ordered hair tests for both the child and his parents to document drug exposure.

A liquid chromatography tandem mass spectrometric (LC–MS/MS) method was then developed to quantify AMI and NOR in hair. After decontamination and segmentation, 20 mg of hair was incubated overnight at 55 °C in methanol (MeOH). The LC–MS/MS method used an online solid phase extraction and the analysis was performed using two transitions per compound. The LOQ and LOD for the two compounds were estimated at 0.0075 ng/mg and 0.005 ng/mg respectively.

All hair segments tested for both parents were negative. For the baby two strands of hair were collected one day after the acute intoxication for the first and 5 weeks later for the second. The first strand was not decontaminated before analysis to avoid losing specimen. The high and relatively homogenous concentrations of AMI (with a range of value from 6.65 to 9.69 ng/mg) and NOR (with a range of value from 7.12 to 8.96 ng/mg) measured suggested that contamination could have occurred. The analysis of the second strand after decontamination allowed to detect AMI and NOR in all hair segments. The obtained values varied between 0.54 and 1.41 ng/mg for AMI and between 1.26 and 4.00 ng/mg for NOR. These results supported the hypothesis of a chronic exposure during several months before hair collection with regular increase. However a single overdose could not be totally excluded. The interpretation of results must take into account the pharmacological and physiological parameters of hair of the children.

1. Introduction

Hair analysis for drugs and drugs of abuse is applied in various settings today, for example in child protection cases, abstinence control programs and workplace drug testing [1].

This alternative matrix offers several advantages highlighting its large window of detection (months) and non-invasive collection. Blood and urine remain the matrices of choice for toxicology analysis in the case of a recent exposure to a drug, whereas hair analysis can enable to show chronic and past consumption. It is possible to perform segmental analysis, which allows the determination of the historic pattern of drug use if the sample is cut as close as possible to scalp on vertex posterior region. Segmental hair analysis allows thus to differentiate a single exposure from a chronic exposure. However, in the case of children there are several difficulties. The lower amount of sample versus adults may interfere with an optimum segmentation and with analysis, especially with decontamination processes. The interpretation of findings must also take account of the differences in physiological and pharmacological parameters of the children's hair versus adult's hair. In particular the porosity is more important in hair of children and can contribute to a contamination by sweat [2–4].

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This paper will present a case of a poisoning of a baby. Hair analysis provided additional information but raise difficulties concerning interpretation of results.

2. Case report

A 6-month-old baby girl was hospitalized in the pediatric emergency initially for an 8% break in her weight curve and a malaise. This baby did not have past medical history or treatment. Clinical signs included generalized hypotonia or hypertonia, paleness, cheeks flush, chewing and systematized movement of the two hands. An electroencephalogram was performed during a crisis identified by the mother and the neurologist concluded to the absence of pathological waves. Furthermore, the classical biological analyses were normal. She went back home after recovering a good appetite and weight increase. But a second hospitalization in intensive care unit happened only 10 days later, with the following clinical signs: coma with mydriasis, respiratory pause, generalized tonic–clonic seizure and fast osteotendinous reflexes. These symptoms were evocative of a pyramidal syndrome. Her condition required: intubation, ventilation and injection of anti-epileptic drugs (Fosphenytoin, Diazepam, Atropine, Ketamine, Propofol, Cisatracurium, Midazolam, Sufentanil). Furthermore, no reaction to light and absence of eye tracking and contact were evocative of blindness neck hypotonia. The ophthalmologic examination showed a bilateral retinal hemorrhage and cerebral IRM revealed subdural hematomas and cortical laminar necrosis. All these clinical signs were strongly evocative of a shaken baby syndrome.

Toxicological screenings of blood and urine were performed using chromatographic techniques and revealed the presence of amitriptyline (AMI) and its active metabolite nortriptyline (NOR). AMI and NOR were quantified in blood using liquid chromatography coupled to a diode array detector (LC-DAD) at 99.4 μg/L and 154 μg/L respectively. This antidepressive drug has no indication in baby, thus there was no reference value and the interpretation of plasma concentrations was difficult. Given the family context, a judge ordered hair tests for both the girl and her parents to document drug exposure: was it an acute intoxication, a one-time exposure or an *in utero* exposure?

The criminal investigation discovered the grandmother and the father had a prescription of amitriptyline. And clinically, the association of cerebral and retina lesions after elimination of other diagnosis, such as congenital and acquired coagulation disorders, cerebral arteriovenous malformation, metabolic diseases (glutaric aciduria, Menkes syndrome), and osteogenesis imperfect, was very evocative of a shaken baby syndrome.

For the baby, a first hair strand was collected one day after the acute intoxication and a second strand was collected 5 weeks later (Fig. 1). For the parents, samples were collected 3 months later.

Fig. 1. Hair specimens collected.

3. Toxicological analyses

3.1. Toxicological screening of blood and urine

Toxicological screenings of urine were performed using liquid chromatography coupled to a diode array detector (LC-DAD) and gas chromatography coupled to mass spectrometry (GC–MS). Because of the small quantity of the collected samples, the screening of blood was realized only using LC-DAD.

3.1.1. Gas chromatography–mass spectrometry analysis

The analyses were performed on an Agilent Technologies 6890N Network GC System combined with an Agilent Technologies 5975 network mass selective detector and an Agilent Technologies 7683 series injector. The entire process, including data collection, was controlled by the Agilent Technologies Chem Station Version Rev.D.02.00.275.

The extractions of the samples were realized using Toxivial A® (Interchim, France) and 1 μL of each extracted sample was injected on to a DB-5 ms UI column (30 mm × 0.25 mm internal diameter; 0.25 μm film thickness) using pulsed splitless injection with an injector temperature of 250 °C [5]. Temperature conditions were as follows: initial temperature of 70 °C for 1 min, increased to 100 °C at 10 °C/min, then increased to 300 °C at 20 °C/min and held for 12 min. The flow of the carrier gas (helium) was maintained at 1 mL/min in constant flow mode. The gas chromatograph interface temperature was held at 315 °C. Electron impact ionization was performed at 70 eV, with an ion source temperature of 230 °C and mass spectra collected from 40 to 600 *m/z*.

3.1.2. Liquid chromatography-diode array detection analysis

A liquid–liquid extraction of the sample was performed. Internal standard solution (30 μL of glafenine at 10 mg/L), 2 mL of carbonate buffer pH 9.2 and 4 mL of dicholoromethane/hexane solution (3/4, v/v) were added to 1 mL of plasma in a glass tube. Then sample was shaken for 10 min and centrifuged 10 min at 2500 rpm, the organic layer was evaporated to dryness under nitrogen. Each extract was reconstituted with 100 μL of phosphate buffer and CH_3CN/H_2O (70/30, v/v) and injected into the chromatograph.

The HPLC system (SHIMADZU *Prominence*) consisted of 2 pumps LC 20AD, a cooled autosampler SIL 20 AC, an integrated column heater and a diode array detector SPD M 20A. For chromatographic separation, a NUCLEOSIL 100-3 C18 column (150 mm × 4.6 mm, Macherey Nagel, Germany) with integrated guard column was used. The eluent consisted of phosphate buffer pH 2.6 (mobile phase A) and CH_3CN/H_2O (90/10, v/v) (mobile phase B). The elution gradient profile used was performed at 1.3 mL/min and it started with 15% of B from 0 to 2 min. Thereafter (2–42 min) the organic fraction B was increased to 75%, then (42–48 min) to 90%. A final equilibration step until 60 min ended the chromatographic run with 15% of B. The injection volume was 50 μL of plasma extracted sample. The analytes were detected at 230 nm.