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Case report

Hair testing in postmortem diagnosis of substance abuse: An unusual case of slow-release oral morphine abuse in an adolescent

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ABSTRACT

Morphine sulfate misuse is essentially observed among regular heroin injectors. To our knowledge, primary addiction to morphine sulfate is exceptional, especially among young adolescents. A 13-year-old girl, with no history of addiction, was found dead with three empty blisters of Skenan® LP 30 mg at her side. Opiates were detected in biological fluids and hair by chromatographic methods. Blood analyses confirmed morphine overdose (free morphine: 428 ng/mL; total morphine: 584 ng/mL) and segmental hair analysis confirmed regular exposure over several months (maximum morphine concentration 250 pg/mg). Suspecting the victim's mother of recreational use of Skenan®, the magistrate ordered analysis of her hair, with negative results. From an epidemiological viewpoint, this case of oral morphine sulfate abuse in an adolescent with no previous history suggests the emergence of a new trend of morphine sulfate consumption. From a toxicological viewpoint, it demonstrates the value of hair testing, which documented the victim's regular exposure and made an important contribution to the police investigation.

1. Introduction

Morphine sulfate is a schedule II opioid under the most recent Controlled Substances Act.[1] In France, morphine sulfate is regulated as a narcotic and prescribed using a special form for a maximum period of 28 days.[2] It is marketed under parenteral forms, immediate-release oral forms and slow-release oral forms (slow-release oral morphine or SROM), which are all indicated for the relief of moderate to severe acute and chronic pain where an opioid analgesic is appropriate. As SROM appears to reduce craving and depressive symptoms, its effectiveness as maintenance therapy for opioid dependence is under debate.[2,3] In some European countries and Australia, SROM is prescribed as an alternative to methadone maintenance treatment in patients who do not tolerate methadone or with inadequate withdrawal suppression.[4–6] In France, SROM can be prescribed in cases of methadone and buprenorphine failure or contra-indications to these substances, when authorized by the medical officer of the health insurance system.[7] But according to the recent Cochrane systematic review, evidence of the effectiveness of SROM for opioid maintenance is insufficient.[3] While the quality of life of patients treated with SROM does not significantly differ from that of those treated with methadone or buprenorphine, side effects (nausea, headache, constipation, insomnia) are more frequent with SROM.

Morphine misuse or abuse is a well-known problem. The effects sought are close to those of heroin: to obtain a high and a sensation of wellbeing.[8] Intravenous and nasal routes are the most common ways of administration.[8] In most cases, morphine sulfate is consumed by heroin injectors and polydrug users with a long history of drug abuse.[6,8] Primary consumption is uncommon. Most users are adults who were prescribed SROM for long periods, or healthcare professionals.[9,10] Cases involving very young adolescents appear to be exceptional.

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In addition, in this case hair testing had a particularly important impact on the course of the police investigation, as it both established the innocence of the victim's mother and led to the dismantling of a drug-trafficking ring.

2. Case report

A 13-year-old girl was found unconscious on the living room sofa by her mother, who called the emergency services at 6.30 pm. The emergency services found the girl in cardiopulmonary arrest. Resuscitation attempts were unsuccessful and death was pronounced at 7.00 pm. According to the mother, the girl had complained of abdominal pain in the morning and had taken a tablet, of which the mother knew neither the name nor the source, in the early afternoon. She then fell deeply asleep and her mother tried to wake her at 6.15 pm. The victim was not receiving any treatment, and according to the mother she had no known history of addictive disorder or suicidal intent.

One capsule and three empty blisters of Skenan LP® 30 mg were found at the girl's side. Careful search of the apartment revealed no other blisters of Skenan LP® or any other opioid. The mother stated that no family member was treated with the drug, and this was confirmed by the family doctor when questioned by police.

The autopsy was carried out the next day and revealed only pulmonary and brain edema. There were no visible injection sites. Specimens were taken for conventional toxicological analysis: heart and femoral blood with NaF 1% as preservative, gastric content, vitreous humor and urine. At the beginning of autopsy, four 32-cm brown hair strands were collected from the posterior vertex region of the scalp. Specimens were stored at +4 °C until analysis, except the hair strands which were stored at room temperature.

Suspecting the mother of consuming Skenan® for recreational purposes, the magistrate ordered analysis of her hair. A 30-cm brown hair strand was collected from the posterior vertex region.

3. Materials and methods

Alcohols (ethanol, 1-propanol, acetone, isopropanol, butanol) were analyzed by gas chromatography with head space injection and flame ionization detector (HS-GC/FID) in femoral blood, urine and vitreous humor. Urinary screening was done by immunochromatography (Nal Von Minden GmbH, Regensburg, Germany) for opioids, amphetamines, cannabinoids, cocaine, methadone, benzodiazepines, antidepressants and barbiturates.

Cardiac blood was screened for drugs of abuse (including opioids, amphetamines and other hallucinogens, cannabinoids, cocaine, GHB/GBL, solvents and benzodiazepines) and pharmaceuticals by routine procedures including liquid chromatography coupled with a diode array detector (HPLC-DAD) and gas chromatography coupled with mass spectrometry (GC-MS).

3.1. Determination of opiates in biological fluids

Opiates (morphine, codeine, diacetylmorphine, 6-monoacetylmorphine, pholcodine and ethylmorphine) were measured in femoral blood, gastric content and urine by GC-MS. Morphine and morphine-d3 (as internal standard, IS) were obtained from LGC Standards (Molsheim, France). β-Glucuronidase from bovine liver, type B-1, was obtained from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Stock solutions of morphine and morphine-d3 were prepared in methanol at 1 mg/mL and stored at −20 °C. The working solutions were diluted with methanol before use for preparation of calibration standards.

For determination of free morphine, 50 μL of IS at 1 ng/μL, 1 mL of dipotassium phosphate 1 M (pH 8.4) and 3 mL of chloroform/isopropanol (95:5, v/v) were added to 1 mL of the specimen to be analyzed. The mixture was shaken for 10 min and centrifuged at 3000 rpm for 3 min. The organic phase was removed and evaporated to dryness under nitrogen. The residue was dissolved in 50 μL of trimethylsilyl (TMS) and heated for 20 min at 70 °C. After cooling, an aliquot of this solution (1 μL) was injected into the GC-MS.

For determination of total morphine, 1 mL of the specimen to be analyzed was incubated at 60 °C for 24 h with 1 mL of acetate buffer (pH 5) containing 30,000 U of β-glucuronidase, and then treated as described for the free fraction. The amount of conjugated morphine (morphine glucuronide) was evaluated by calculating the difference between total and free morphine. The chromatographic conditions have been previously described.[11] The retention time (rt) and fragment ions for identification (m/z) were as follows: morphine (rt: 12.45 min, m/z: 429, 414, 401) and morphine-d3 (rt: 12.42 min, m/z: 432, 417, 404).

3.2. Determination of opiates in hair

The 32-cm hair strands of the victim were cut into five segments. The first four segments measured 6 cm each, the last 8 cm. The period analyzed extended from June 2008 to January 2011. The hair strand of the mother was cut into three segments of 3 cm each. The period analyzed extended from April 2009 to October 2011. The distal segment (21 cm) was not analyzed.

Opiates were determined in hair by liquid chromatography-tandem mass spectrometry (LC-MS/MS) after triple liquid–liquid extraction as follows: each hair segment was washed in methanol and then in dichloromethane. Twenty mg of finely cut hair was weighed. IS was added together with 1 mL of HCl 0.1 M for incubation overnight at 56 °C. Phosphate buffer pH 8.4 was added for neutralization and alkaline extraction with dichloromethane-isopropanol-heptane. The organic phase was then acidified with HCl 0.2 M for a second extraction. The aqueous phase was isolated and NaOH 1 M and saturated phosphate buffer pH 8.4 were added for a third extraction with dichloromethane. The final organic phase was evaporated and diluted with formiate buffer 2 mM pH 3.0 for injection onto LC-MS/MS apparatus (TSQ Quantum, Thermo Fisher Scientific Inc., Villebon-sur-Yvette, Courtaboeuf, France). The following ions were used: morphine: parent ion m/z 286.1 and daughter ions m/z 152, 153, 181; morphine-d3: parent ion m/z 289.1 and daughter ions m/z 152, 153, 181.

4. Results

4.1. Toxicological analysis

Toxicological analysis showed the presence of morphine in all specimens. Morphine concentrations in the victim's biological fluids are shown in Table 1. It is important to note that only morphine was identified in these specimens, and testing for other

Table 1
Morphine concentrations in the victim's biological fluids.

	Femoral blood (ng/mL)	Urine (ng/mL)	Gastric content (ng/mL)
Unconjugated morphine	428	11,500	87,750
Total morphine	584	13,800	Unavailable
Conjugated morphine	156	2330	Unavailable