CASE REPORT

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Fatal Intoxication Following Self-Administration of a Massive Dose of Buprenorphine

ABSTRACT: Several drug packages, including Subutex® (high-dose buprenorphine, as sublingual tablets) boxes, were found near the corpse of a 25-year-old male drug addict, who apparently had committed suicide. The autopsy revealed a fatal respiratory depression. The toxicological investigations concluded that death resulted from massive burprenorphine intoxication. The determination of buprenorphine (BU) and norbuprenorphine (NBU) in all biological specimens was performed by liquid chromatography-electrospray-mass spectrometry (LC-ES-MS) after hydrolysis (for solid tissues), deproteinization of the matrices, and solid-phase extraction of the compounds. Exceptionally high concentrations of BU and NBU were found in blood (3.3 and 0.4 mg/L, respectively), urine (3.4 and 0.6 mg/L), bile (2035 and 536 mg/L) and brain (6.4 and 3.9 μg/g). The high concentration of BU (899 mg/L) and the absence of NBU in gastric liquid suggested oral intake. High concentrations of amino-7-flunitrazepam, the main metabolite of flunitrazepam, were also found in blood, urine and gastric liquid. This benzodiazepine may have been a co-factor in the toxic effects of BU.

KEYWORDS: forensic science, buprenorphine, flunitrazepam, LC/ES/MS

Buprenorphine (BU), a derivative of thebaine, is an outstanding narcotic agent. This semisynthetic opioid acts as an agonist-antagonist for morphine receptors: it expresses agonist properties on μ-receptors and antagonist properties on κ-receptors, and a high affinity for both types. Its behavior is that of a pseudo-irreversible ligand, as it shows a very slow dissociation from opiate receptors. Consequently, BU exerts a long activity (twice as long as that of morphine) and is weakly antagonized by naloxone (1,2). Pharmacokinetically, BU is characterized by a weak oral bioavailability and low therapeutic concentrations, owing to its high lipid solubility. Its main metabolite is norbuprenorphine (NBU), which is phar-

macologically active but normally does not cross the blood-brain barrier. BU and NBU are further glucuro-conjugated and their glucuronides are mainly excreted in bile (3,4).

Buprenorphine has been widely used for about 20 years in the treatment of moderate-to-severe pain under several trade names. Under the name Subutex® (high-dosage formulation), buprenorphine became available in 1996 in France for the maintenance treatment of heroin abuse, as an alternative to methadone. Contrary to the latter, delivered on a daily basis in specialized centers, Subutex® sublingual tablets (0.4, 2 and 8 mg) can be prescribed by any general practitioner for the ambulatory treatment of opioid dependence, and the entire dose for up to 28 days can be delivered by any pharmacist. Today, this drug is largely used and abused in France. In spite of recommendations for "medical, social and psychological care" accompanying the prescription and the treatment, this substitutive therapy has resulted, directly or indirectly, in a number of intoxication cases, sometimes fatal, generally due to deviations and misuses like intravenous injections or association with other psychotropic drugs (mainly benzodiazepines) (5,6).

We report here a fatal intoxication representative of this worrying situation, and illustrate the efficiency of liquid chromatography-electrospray-mass spectrometry (LC-ES-MS) for identifying and quantitating BU and NBU in various biological specimens.

Case History

A 25-year-old unemployed man, known to be addicted to heroin, was found dead in his hotel room. The bedroom was not untidy, and the door was locked from the inside. A handwritten suicide note and several drug packages (among which Subutex® boxes) were found near his body.

No suspect lesion or wound of traumatic origin, evocative of external aggression, was disclosed during corpse examination. The autopsy revealed a congestion of the two lungs without macroscopic traumatic lesion of the respiratory tract. Liver and spleen were congestive too. A cerebral oedema associated to a dilatation of the cortex vessels was observed. These findings were consistent with a major respiratory depression probably at the origin of death.

Several biological samples (blood, urine, vitreous humour, lung, liver, kidney, brain, heart and gastric content) were collected for toxicological investigations.

Material and Methods

Opiates, amphetamines, cannabinoids and benzoylecgonine were screened for in urine using fluorescence polarization immunoassays. A wide screening of drugs and toxicants in biological fluids was performed using both high-performance liquid chromatography coupled to a diode array detector (HPLC-DAD) and gas chromatography/mass spectrometry (GC/MS). More selective analyses for several classes of therapeutic drugs—drugs of abuse, alkaloid poisons, mineral toxicants—were carried out with various dedicated methods using HPLC-DAD, LC-ES-MS, GC with thermoionic or flame ionization detection, GC/MS, atomic absorption spectrometry, etc. Benzodiazepines were specifically screened for and quantitated using a dedicated HPLC-DAD method.

BU and NBU were determined in biological samples using a previously reported method (7,8), based on enzymatic hydrolysis of solid tissues, deproteinization of the hydrolysates as well as of liquid samples, followed by a solid-phase extraction and a chromatographic separation on a reversed-phase column (C18 Nucleosil, 5 μm, 150 × 1 mm inside diameter) using a mixture of 2 mM ammonium formate buffer (pH 3) and acetonitrile (70/30, v/v) as mobile phase (flow rate of 40 μL/min). The mass spectrometric detection was performed in the selected ion monitoring mode after in-source collision-induced dissociation, using an API 100 spectrometer (Sciex, Foster City, Canada) equipped with an electrospray-type Ionspray® ionization device. One quantitation ion and two confirmation ions were selected for each compound.

Results and Discussion

The positive results of the analytical investigations are presented in Table 1. BU concentrations were surprisingly high in all the samples. These levels were not only much higher than the therapeutic range (9,10), but were also higher than those previously reported in buprenorphine related deaths: Tracqui et al. (6) reported concentrations of BU ranging from 1.1 to 29.0 μg/L in blood, from 4 to 1033 μg/L in urine, from 575 to 72 600 μg/L in bile, and from 0.007 to 0.15 μg/g in brain. Likewise, in the present case, the highest concentrations of BU were found in bile and brain, which could be explained by the biliary excretion and the high lipid solubility of the drug, respectively (11,12).

Moreover, the presence of BU and the absence of NBU in gastric content were evocative of a recent oral intake inasmuch as no syringe was found near the corpse and no injection traces could be detected on body examination, whereas most of the fatal intoxications previously reported were by the intravenous, epi- or peridural routes (6,13,14).

High levels of NBU were also found in all the samples, except gastric content. The NBU/BU concentration ratio ranged from 0.1 to 0.2 in blood, urine and bile. In spite of the lack of postmortem kinetic data, these ratio values suggest a short delay between BU administration and death. The high NBU brain concentration found is apparently contradictory with the concept, generally admitted, that NBU does not cross the blood-brain barrier. Moreover, the NBU/BU ratio in brain (0.6) was higher than in biological fluids. Two hypotheses can be put forward to explain this finding: (1) NBU came from the metabolism of BU in brain, but this would mean that this metabolism was more intense than in the other organs, or continued postmortem for a longer time in the brain than in the other organs; (2) NBU did cross the blood-brain barrier and accumulate in the brain owing to its lipid solubility and above all to the importance of the intoxication.

High concentrations of 7-amino flunitrazepam (7AF), a flunitrazepam metabolite, were measured in blood, urine and gastric liquid. The absence of flunitrazepam could be partly due to in vitro degradation of this benzodiazepine during sample storage, since it has been shown that flunitrazepam is totally degraded within 24 h at ambient temperature or at +4°C in the dark (15,16). Moreover, this in vitro degradation mainly produces 7AF through reduction of the nitro- into an amino-moiety. The high concentration of 7AF in gastric liquid suggests a recent ingestion of flunitrazepam, possibly concomitant with that of BU. Moreover, the high blood and urine concentrations of 7AF probably correspond to a high dose of flunitrazepam. Though it is not possible to evaluate the proper toxic effects of such an exposure (17), flunitrazepam may have major BU toxic effects. Indeed, this association may induce severe respiratory depression, as suggested by its finding in the majority of BU intoxication cases linked to substitution (5,6) and as previously noted in anaesthesiology (13,14). 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (a nonpsychoactive metabolite of Δ^9-tetrahydrocannabinol) was found only in urine, at a low concentration. It suggests that Δ^9-tetrahydrocannabinol administration dated back from more than 24 h before death and could not be linked to major toxicological effects.

The presence of ethanol in gastric liquid can be put on the account of postmortem production, owing to its absence in vitreous humour and blood.

Conclusion

According to the toxicological expertise, the coroner's conclusion was BU fatal intoxication, probably following oral intake, potentiated by the association with a benzodiazepine. Exceptionally

TABLE 1—*Toxicological data.*

Substances	Biological Medium				
	Blood	Urine	Bile	Gastr. Liq.	Brain
7-amino flunitrazepam	1 251 μg/L	4 943 μg/L	…*	28 671 μg/L	…
Nor 11-Δ^3-tetrahydrocannabinol-9 carboxylic acid	ND†	1.9 μg/L	…	…	…
Buprenorphine	3 276 μg/L	3 397 μg/L	2 035 mg/L	899 mg/L	6.39 μg/g
Norbuprenorphine	399 μg/L	624 μg/L	536 mg/L	ND	3.92 μg/g
Ethanol	ND	ND	…	1.04 g/L	…

* … = Analysis not performed.
† ND=Not detected.

and surprisingly high concentrations of BU were revealed in various fluids, as well as in the brain, using LC-ES-MS. The part played by benzodiazepines in the toxicity of buprenorphine requires further experimental studies.

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