Fatal Overdosage with Nefopam (Acupan®)

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Abstract

This paper presents a fatality due to massive, intravenous self-administration of nefopam (Acupan), a non-opiate central analgesic, in a 37-year-old female. Nefopam was measured in various postmortem samples by means of high-pressure liquid chromatography coupled to mass spectrometry via an ionspray interface. Heart blood concentration was 4.38 pg/mL and exceeded by approximately 30 times the highest therapeutic levels with the usual reservations concerning possible postmortem redistribution. This is only the third case of death following nefopam overdose reported in the literature.

Case History

A 37-year-old Caucasian female, who had worked as a nurse in a medical rehabilitation unit, was found lying on the floor dead at home. The body was markedly putrefied (estimated time since death: 7–10 days in midseason) and covered with numerous fly larvae. No signs of violence were observed. Fifteen broken ampules of Acupan (equivalent to 300 mg nefopam) were found near the body, together with an used syringe equipped with an i.v. needle. Police inquiry revealed that the deceased had a past history of multiple-drug addiction.

Autopsy findings were unremarkable, excepted for the presence of typical marks of venopuncture at the right antecubital fossa. All viscera were putrefied, and the lungs displayed marked signs of congestion (weight: right 857 g, left 616 g). Macroscopically, there was no evidence of any disease that lowering the seizure threshold (e.g., tricyclics) (1,10,11). Deaths attributed to nefopam seem exceptional. This paper presents a fatality involving a massive, self-administration of Acupan by the i.v. route, in which the drug could be measured at high levels in various postmortem samples by means of high-pressure liquid chromatography coupled to mass spectrometry (HPLC-MS).

Introduction

Nefopam (3,4,5,6-tetrahydro-5-methyl-1-phenyl-lH-2,5-benzoxazocine, MW = 253.34, Figure 1) is a non-opiate central analgesic available for about 20 years in most Western European countries under the tradenames Acupan or Ajan®. Although its mechanism of action has not been fully explained, this drug was found effective for the control of mild to moderate pain, especially of postoperative origin (1–5); at equivalent dosages, nefopam is approximately 10 times more potent than aspirin, 2 to 3 times more potent than pethidine, equipotent to pentazocine, and 2 to 3 times less potent than morphine (1,6–9). Usual doses are 30 to 90 mg per os three times daily, or 20 mg by intramuscular (i.m.) or slow intravenous (i.v.) injection repeated every 4 to 6 h if necessary; maximum recommended doses per os and parenterally are 300 and 120 mg/24 h, respectively (1,10).

Nefopam is generally well-tolerated, the most frequent side-effects being nausea, sweating, dry mouth, tachycardia, and in some studies sedation. Convulsions have been occasionally reported; thus, the drug is contraindicated in epileptic patients and should be used cautiously in association with drugs lowering the seizure threshold (e.g., tricyclics) (1,10,11). Deaths attributed to nefopam seem exceptional. This paper presents a fatality involving a massive, self-administration of Acupan by the i.v. route, in which the drug could be measured at high levels in various postmortem samples by means of high-pressure liquid chromatography coupled to mass spectrometry (HPLC-MS).

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Figure 1. Chemical structure of nefopam.
might have caused, hastened, or facilitated the death. Microscopic examination was not performed. Samples taken for toxicological analyses included heart blood (peripheral blood was no longer available because of the stage of putrefaction), liver, kidney, lung, myocardium, and fly larvae (approximately 100 specimens collected from various areas at the outer surface of the body, total weight 3.1 g).

**Toxicological Analyses**

The determination of nefopam in autopsy samples was carried out using an adaptation of a general purpose HPLC–MS procedure previously described for other analytes (12–15). Briefly, postmortem blood was extracted at pH 9.5 by 5 mL of chloroform/2-propanol/n-heptane (25:10:65, v/v) after addition of 2 µg of prazepam as internal standard (IS). Viscera and fly larvae were first homogenized (one-part tissue in four-parts deionized water, w/v) using an IKA Ultra-Turrax homogenizer (IKA, Staufen i. Br., Germany), then underwent the same extraction protocol. After evaporation, dry extracts were resuspended in 30 µL methanol, from which 2 µL was injected onto a 4-µm NovaPak (Waters, Milford, MA) C18 column (150 × 2.0-mm i.d.) equipped with a 5-µm Opti-Guard™ C18 (Interchim, France) guard cartridge (15 × 1.0-mm i.d.). Each chromatographic run was performed with a binary, linear A/B gradient where solvent A = acetonitrile + trimethylamine (10 µg/mL) and solvent B = 2mM NH₄COOH, pH 3.0 buffer (solvent A 80 to 90% in 9 min, then back to 80% at 10 min). The flow rate was 200 µL/min with a post-column split of 1:3. Under these chromatographic conditions, the average retention times of nefopam and the IS were 4.22 and 3.26 min, respectively. The detection was achieved by a PerkinElmer Sciex (Foster City, CA) API-100 MS equipped with a pneumatically assisted electrospray (= ionspray, ISP) interface. The system was operated in the positive ionization mode with voltages of +4.5 kV, +30 V and +2.6 kV applied to the sprayer, ion sampling orifice (OR) and electron multiplier, respectively. MS data were collected as either total ion current (TIC) or selected ion monitoring (SIM) at m/z 254 and 181 for nefopam and 325 for the IS. Nefopam quantitation was done by computing peak-area ratios (nefopam/IS) of the sample extracts analyzed in SIM (m/z 254/m/z 325) and by comparing them with a six-point

![Figure 2. HPLC–MS chromatogram (SIM mode; m/z 254+181+325, OR = + 30 V) obtained from the postmortem blood (100 µL). Peak A (3.24 min): prazepam, internal standard; Peak B (4.20 min): nefopam, concentration 4.38 µg/mL. Inset: Positive-ion ISP mass spectrum of Peak B, obtained by reprocessing the blood extract in TIC mode (m/z 100–300).](https://academic.oup.com/jat/article-abstract/26/4/239/515555)
standard curve previously constructed by assaying drug-free blood spiked with the analyte at concentrations of 2, 5, 20, 50, 200, and 500 ng/mL.

In addition to nefopam specific analysis, a complementary screening of the postmortem blood was performed using fluorescence polarization immunoassay (FPIA) on the Abbott ADx analyzer (benzodiazepines, barbiturates, tricyclics, opiates, cocaine, cannabinoids, amphetamine derivatives), gas chromatography–flame-ionization detection (GC–FID) (ethanol), and gas chromatography–mass spectrometry (GC–MS) (pharmaceuticals, drugs of abuse, usual organic solvents).

**Results and Discussion**

Since the late 1970s, several procedures have been proposed for the determination of nefopam in biological fluids, most of them involving GC coupled to FID (16,17), nitrogen-phosphorus (GC–NPD) (18), or MS (19) detection. Alternatively, a method based upon liquid chromatography with electrochemical detection (HPLC–ECD) proved highly sensitive, although hampered by the complexity of sample pretreatment (a combination of liquid– and solid-phase extraction) (20). The original HPLC–MS procedure described herein was found to provide a valuable compromise between the ease of sample preparation (a single-step liquid–liquid extraction without prior derivatization), a good sensitivity in the low nanogram per milliliter range, and an excellent specificity suitable for medicolegal applications. Validation of this method was realized using drug-free whole blood samples (1 mL each) spiked with nefopam at various concentrations. The six-point standard curve of calibration showed a good linearity \( r = 0.991 \) over the concentration range tested (2–500 ng/mL) with an equation of \( y = 164.9x - 9.3 \) (\( y = \) nefopam concentration in ng/mL; \( x = \) nefopam area/IS area). The extraction recovery was 85.5 ± 4.3% on blood loaded at 200 ng/mL (six determinations). This was also measured on samples of human liver, kidney, lung, and myocardium, first homogenized in deionized water then spiked to contain nefopam at 40 ng/g (equivalent to 200 ng/g tissue; samples were taken at autopsy of a traffic accident victim, after postmortem blood had been checked negative for nefopam and prazepam): the extraction recoveries (six determinations on each matrix) were 61.5 ± 5.3% (liver), 68.7 ± 6.6% (kidney), 70.1 ± 5.9% (lung), and 63.0 ± 7.1% (myocardium). Although lower than in blood, these recoveries were found reasonable. In addition, extraction recoveries for the IS prazepam were also found reduced and not significantly different, in each matrix, from those obtained for nefopam; thus the calibration curve and quantitation method developed from blood standards were considered appropriate to the analysis of viscera homogenates. Within-run accuracy and precision, measured at 200 ng/mL (six replicates) were 6.8 ± 1.7% and 9.1 ± 1.4%, respectively. The LOD (three times the background noise in SIM at \( m/z \) 254) was 0.5 ng/mL for 1 mL analyzed, and the LOQ was set at 2 ng/mL. In case of concentrations exceeding the upper point of the calibration curve (500 ng/mL), samples were reassayed after 10-fold dilution with deionized water and this operation was reiterated until the measured concentration falls within the calibration range.

Using this analytical procedure, nefopam could be identified in all autopsy samples related to our observation. Figure 2 represents the HPLC–MS chromatogram (SIM mode; \( m/z \) 254 + 181 + 325) of the postmortem blood extract, together with the positive-ion, ISP mass spectrum of nefopam (this was obtained by reprocessing the same sample in TIC mode, \( m/z \) 100 to 300). At moderate values of the OR potential (0 to + 50 V), nefopam typically displays a base peak at \( m/z \) 254 (corresponding to the protonated analyte \([M + H]^+\)), a major fragment at \( m/z \) 181 that can be used as the confirmation ion, and a much smaller but constant fragment at \( m/z \) 166. Except for a shift of 1 mass unit due to protonation, this fragmentation pattern partially fits with that previously observed when applying electron impact mass spectrometry on underivatized nefopam (peaks at \( m/z \) 165, 180, 253) (21,22). The concentrations of nefopam in the samples assayed are listed in Table I. With the exception of a low blood ethanol concentration at 0.012 g/dL (hard to interpret because of the stage of putrefaction), no other pharmaceuticals or toxicants could be identified by the complementary screening. Interestingly, the Abbott® immunoassay performed on blood returned positive for benzodiazepines, but was not confirmed by the GC–MS screening subsequently achieved. Considering that similar false positives (presumably due to a structural resemblance to oxazepam) have recently been shown to affect the Syva Behring® EMIT d.a.u. benzodiazepine assay (23), this suggests that nefopam may be able to interfere some with benzodiazepine immunoassays from various manufacturers.

### Table I. Fatalities Involving Nefopam: Review of the Literature

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Gender, age</th>
<th>Circumstances of Death</th>
<th>Nefopam Concentrations (µg/mL or µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(29)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Urwin and Smith (1999)</td>
<td>F, 38</td>
<td>Voluntary ingestion of nefopam (+ dihydrocodeine) tablets. Death at ICU 3 days after admission.</td>
<td>Plasma 4.3 (admission)*</td>
</tr>
<tr>
<td>(30)</td>
<td></td>
<td></td>
<td>Plasma 0.6 (day 1)</td>
</tr>
<tr>
<td>Present observation</td>
<td>F, 37</td>
<td>Voluntary self-injection of nefopam by the i.v. route. Found dead at home.</td>
<td>Blood 4.38, Liver 59.12, Kidney 14.57, Lung 44.07, Myocardium 14.87, Fly larvae 0.88</td>
</tr>
<tr>
<td>(2001)</td>
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* Dihydrocodeine 5.9 µg/mL.
According to the literature (1,10,21), nefopam is readily absorbed from the gastrointestinal tract after oral administration. A single, therapeutic dose of 90 mg produces peak plasma concentrations of 0.073 to 0.154 µg/mL at 1 to 3 h following the intake. Time of peak concentration following an i.m. dose is approximately 1.5 h. Nefopam distributes well to the entire body (volume of distribution 410 to 447 L [24]) and is present in the milk of nursing mothers at levels equivalent to those in plasma (25). The drug is extensively metabolized, less than 5% of a dose being excreted unchanged in the urine. Three metabolites (all inactive) have been identified: desmethylnefopam, nefopam glucuronide, and nefopam N-oxide. The plasma half-life of elimination ranges from 3 to 8 h (mean 4 h) after an oral or i.v. dose.

Data on the toxicology of nefopam in humans or animals are relatively few. Dogs given 40 to 80 mg/kg/day mainly show stimulation of the CNS with excited behavior and seizures (26). LD₅₀ values in various animal species (mouse, rat, dog) range from 100 to 200 mg/kg orally, 30 to 57 mg/kg i.m., and 20 to 45 mg/kg i.v. (1,27,28). To our knowledge, there have been only two cases of fatal nefopam overdosage previously reported in the literature (Table I) (29,30). Both followed a voluntary, massive ingestion of nefopam tablets and were clinically well-documented. The symptoms displayed by the patients were remarkably close: deep coma with convulsions, tachycardia, and by (hypoxic?) brain damage in the latter. Clinical features involving partial opioidergic component (34-36), and at least attempt, or of an accidental overdosage in a nefopam abuser. Only two cases of fatal nefopam overdosage previously reported in the literature (Table I) (29,30). Both followed a voluntary, massive ingestion of nefopam tablets and were clinically well-documented. The symptoms displayed by the patients were remarkably close: deep coma with convulsions, tachycardia, and by (hypoxic?) brain damage in the latter. Clinical features involving partial opioidergic component (34-36), and at least attempt, or of an accidental overdosage in a nefopam abuser. Although its addiction liability is generally considered weak (1,31-33), nefopam is believed to exert its analgesic action through central (spinal and/or supraspinal) mechanisms involving partial opioidergic component (34-36), and at least two observations of chronic abuse have been reported in the literature (37,38). In the present casework, the postmortem blood concentration (4.38 µg/mL) exceeded the highest therapeutic levels by approximately 30 times. The value obtained from heart blood (peripheral blood was not available) should be considered with some reservations because of the frequent biases related to postmortem redistribution (especially with compounds exhibiting large volumes of distribution). This finding was, however, associated with high concentrations in the viscera, leaving few doubts on the reality of the overdosage. Lower concentrations were also found in the fly larvae sampled on the body; the interest of such biological specimens as qualitative markers of drug exposure has been discussed elsewhere (39,40). Finally, the medical examiner ruled the cause of death to be a massive nefopam overdosage and returned no conclusion on the manner of death.

References


