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Fatal tolperisone poisoning: Autopsy and toxicology findings in three suicide cases

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ABSTRACT

Tolperisone (Mydocalm®) is a centrally acting muscle relaxant with few sedative side effects that is used for the treatment of chronic pain conditions. We describe three cases of suicidal tolperisone poisoning in three healthy young subjects in the years 2006, 2008 and 2009. In all cases, macroscopic and microscopic autopsy findings did not reveal the cause of death.

Systematic toxicological analysis (STA) including immunological tests, screening for volatile substances and blood, urine and gastric content screening by GC–MS and HPLC–DAD demonstrated the presence of tolperisone in all cases. In addition to tolperisone, only the analgesics paracetamol (acetaminophen), ibuprofen and naproxen could be detected. The blood ethanol concentrations were all lower than 0.10 g/kg. Tolperisone was extracted by liquid–liquid extraction using *n*-chlorobutane as the extraction solvent. The quantification was performed by GC–NPD analysis of blood, urine and gastric content. Tolperisone concentrations of 7.0 mg/l, 14 mg/l and 19 mg/l were found in the blood of the deceased.

In the absence of other autopsy findings, the deaths in these three cases were finally explained as a result of lethal tolperisone ingestion. To the best of our knowledge, these three cases are the first reported cases of suicidal tolperisone poisonings.

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1. Introduction

Tolperisone, 2-methyl-1-(4-methylphenyl)-3-(piperidinyl)-1-propanone (chemical structure in Fig. 1), was first described as a centrally acting muscle relaxant by Porszasz et al. in 1961 [1]. In contrast to peripheral muscle relaxants and neuromuscular-blocking drugs such as curare derivatives, centrally acting muscle relaxants, also referred to as spasmolytics, such as carisoprodol, baclofen, clonidine, diazepam, tetrazepam and gabapentin, act at the level of the cortex, brain stem or spinal cord. Tolperisone, eperisone, inaperisone, lanperisone and silperisone are the best known drugs within the group of the piperidine derivatives [2]. Tolperisone is generally used as a racemic mixture. After injection of the active stereoisomer, tolperisone is rapidly converted into the racemate [3].

Tolperisone inhibits voltage-dependant Na^+-channels in the brain-stem and, as a result, inhibits pathologic mono- and polysynaptic reflex activity [4,5]. Because it has fewer sedative side effects than other muscle relaxants, psychomotoric impairment (driving, operation of machines) is strongly reduced. Possibly critical side effects of tolperisone were reported by Ribi et al. in four patients at the University Hospital of Geneva between November 2001 and March 2003; these side effects included vasodilatation, hypotension and anaphylactic reactions [6].

A typical treatment regimen for tolperisone is 3×50 mg per day (max. 600 mg), ingested orally. The oral bioavailability is low, approximately 20%. The maximum plasma concentrations, 64–785 ng/ml (average 297 ng/ml), were measured after oral administration of 450 mg tolperisone [7]. Tolperisone is mainly metabolized by CYP2D6. The main metabolites are hydroxymethyl-tolperisone (by hydroxylation) and hydroxy-tolperisone (by reduction). The elimination half-life varies from 43 min to 174 min depending on the application [7,8]. The biphasic elimination after administration of a single dose is completed after 24 h.

To the best of our knowledge, no data about toxic and lethal tolperisone concentrations have been published in the scientific literature. Only one case of suicidal ingestion of a mixture of ten drugs including tolperisone, yielding a tolperisone blood concentration of 3.36 mg/kg, was found in the literature [9].

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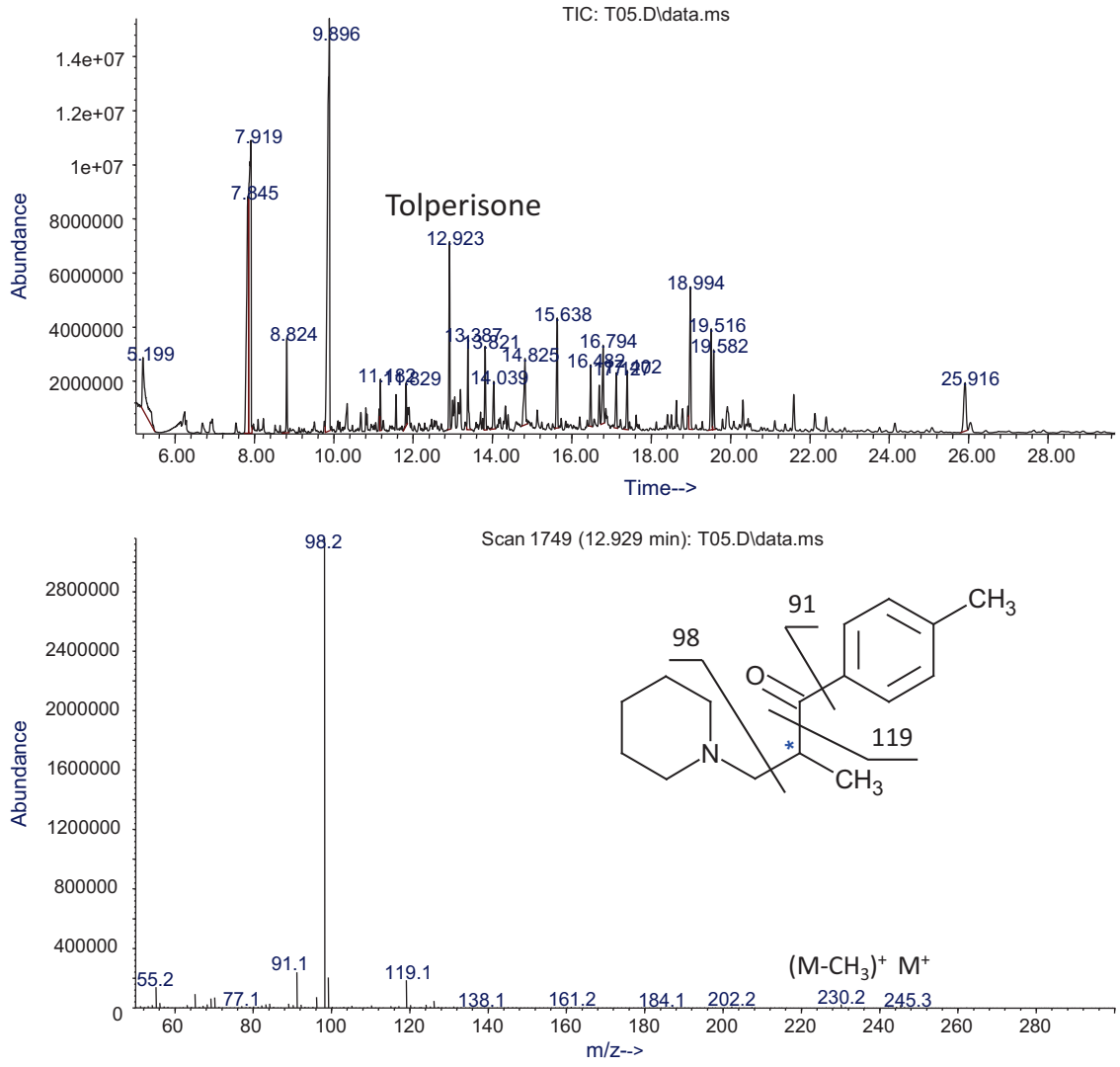

Fig. 1. Total ion chromatogram (TIC) and MS spectra of tolperisone in the blood extract of case 2 and corresponding library spectra (MPW2007).

2. Case histories

Case 1: A 14-year-old female was found dead at home by her relatives in 2006. No sign of struggle was observed at the place of death. According to the police investigation, the girl had attempted suicide using drugs four months earlier.

Case 2: A 20-year-old female was found dead in her boyfriend's home in 2008. A suicide note and several drugs, among them tolperisone, were discovered at the place of death.

Case 3: A 41-year-old female was found dead in the summer of 2009 in a forest. Three years before, she had obtained a Mydocalm® prescription because of psychosomatic pain. No sign of crime or drug ingestion was noted at the place of death.

The external examinations of the bodies were unremarkable, and the macro- and microscopic autopsy findings all showed visceral congestion and lung edema. The autopsy findings did not reveal the cause of death in any of the three cases.

3. Material and methods

3.1. Samples

Biological fluids and tissue samples of the three autopsy cases were routinely taken during autopsy at the University Center of Legal Medicine of Lausanne and were immediately frozen and kept until analysis at −21 °C. The autopsies took place 40, 60 and 48 h, respectively, after the death. Post-mortem serum was obtained by direct centrifugation of peripheral femoral blood during autopsy.

Blood, urine and gastric contents were submitted to a systematic toxicological analysis according to our routine procedure [10].

3.2. Reagents

Tolperisone hydrochloride, paracetamol, naproxen sodium, ibuprofen sodium, and 5-(p-methylphenyl)-5-phenylhydantoin (MPPH) were obtained from Sigma–Aldrich (Buchs, Switzerland). The internal standard (I.S.) methaqualone was obtained from Lipomed (Arlesheim, Switzerland). Sodium chloride (p.a.), potassium dihydrogen phosphate (p.a.), ammonium chloride (p.a.), ammonium hydroxide (25%), acetonitrile (HPLC grade), ethyl acetate (GC grade), methanol (GC grade), *n*-chlorobutane (GC grade), and toluene (GC grade) were purchased from Sigma–Aldrich (Buchs, Switzerland).

3.3. Solutions

Stock solutions were prepared at 1 mg/ml in methanol for tolperisone and methaqualone and at 10 mg/ml for paracetamol, ibuprofen, naproxen and MPPH. Working solutions of tolperisone and the I.S. were at 10 μg/ml in methanol. For quality controls, second working and stock solutions of tolperisone with the same concentrations as above were prepared.

3.4. Extraction procedures

Blood, post-mortem serum, urine, cerebrospinal fluid, bile and gastric contents were extracted according to the following procedures: to 0.5 ml of each liquid sample (for bile and gastric content, 0.01 ml of the homogenized sample), 100 μl of

the I.S. solution at 10 μg/l (final concentration, 1000 μg/l) and 2 ml of saturated ammonium chloride buffer (pH 9.5) were added. *n*-Chlorobutane (4 ml) was used as the extraction solvent because of its good efficiency in an extraction test [11]. After a 15 min extraction on a horizontal shaker at 200 strokes/min, the samples were centrifuged for 5 min at 4000 rpm. The upper layer was collected and evaporated to dryness at 30 °C under a gentle stream of nitrogen. The residue was dissolved in 100 μl of toluene. Liver and brain tissues (each 0.5 g, to which was added 1 ml of 0.9% sodium chloride) were homogenized with a Warring blender (Bender & Hobein, Zurich, Switzerland), sonicated for 15 min with 2 ml of saturated ammonium chloride buffer (pH 9.5) and finally extracted using the same method used to extract the liquid samples.

Paracetamol was extracted from 0.5 ml of whole blood adjusted to pH 9.5 with 2 ml of saturated ammonium chloride buffer (pH 9.5) by liquid-liquid extraction (LLE) with 3 ml ethyl acetate. Ibuprofen and naproxen were extracted from 0.5 ml of whole blood buffered at pH 2.5 with 0.5 M potassium dihydrogen phosphate by LLE with 4 ml *n*-chlorobutane. MPPH at a concentration of 5 μg/l was used as internal standard in all cases. All substances were measured and quantified by HPLC–DAD according to our validated laboratory procedure. The methods had been validated prior (data not published).

3.5. Instrumentation and conditions

An Agilent 6890 Network GC-System (Palo Alto, USA) equipped with a nitrogen-phosphorous detector and a 7683 Series injector controlled by G1701 DA MSD ChemStation software was used for quantitative measurements of the blood, urine and tissue samples. Separation was performed on an Agilent-XLB capillary column (30 m × 0.25 mm i.d. × 0.25 μm). One microliter was injected onto the column in splitless mode with an injector temperature at 260 °C. Helium 5.0 at a flow rate of 1.5 ml/min was used as the carrier gas. The oven temperature was kept at 100 °C for 1 min, increased to 180 °C at 20 °C/min and then to 310 °C at 10 °C/min (held at 310 °C for 12 min). The detector temperature was 300 °C. The flow rates of hydrogen, air, and the make-up gas (nitrogen) were adjusted to 2.0, 60.0, and 30.0 ml/min, respectively.

An Agilent 1100 HPLC-DAD system was used for paracetamol, ibuprofen and naproxen analysis. Thirty microliters of the sample extract in the mobile phase was injected into a short CC 8/3 Nucleodur 100-5 C8 ec enrichment precolumn (Macherey-Nagel) at a flow rate of 0.5 ml/min. The separation of the analytes was performed on a CC 250/3 Nucleodur 100-5 C8 ec column (Macherey-Nagel). The elution program started with 100% solvent A [phosphate buffer (0.01 M, pH 2.3) – acetonitrile 95:5, v/v)], changed linearly to 70% solvent B [phosphate buffer (0.01 M, pH 2.3) – acetonitrile 30:70, v/v)] over 20.5 min, held at 70% solvent B for 8.5 min, and then returned to the initial conditions over 3 min. After 3 min of equilibration, the next run was started. For data acquisition by the DAD, two wavelengths were chosen: 246 (paracetamol) and 220 nm (ibuprofen and naproxen).

3.6. Validation

Tolperisone was quantified in blood using a standard addition procedure, which is carried out in our laboratory in cases when analytes have to be quantified less than once a year. For each blood sample, two samples of the same blood fortified with 5.0 or 20 mg/l tolperisone were analyzed identically. The resulting calibration curves ($r^2 > 0.99$) were used for the calculation of the tolperisone concentration.

Additionally, external calibration was performed from 0.1 to 10 mg/l at seven different concentrations (0.1, 0.2, 0.4, 1.0, 2.0, 5.0 and 10 mg/l) in drug-free whole bovine blood (EDTA stabilized). Six tolperisone free blood extracts were injected on the GC–NPD in order to test the system's selectivity.

Because no commercial quality control sample was available, an internal quality control sample based on an independent stock solution of 1.0 mg/l was prepared and analyzed in the same way. The relative standard deviation from the theoretical concentration (based on the linear regression of the seven-point calibration) was less than 15% for these samples. Calibration was always linear ($r^2 > 0.99$) and was used to estimate the tolperisone concentrations in blood and in all liquid and tissue samples. The deviation of the tolperisone concentration based on external calibration was less than 6% compared to the concentration obtained by standard addition.

4. Results and discussion

The results of the systematic toxicological analysis (STA) revealed in all three cases the presence of tolperisone. As an example, Fig. 1 shows the total ion chromatogram (TIC) for the urine extract of case 2 with the MS-spectra of tolperisone. Tolperisone and its metabolite hydroxyl-tolperisone were identified by comparison of the obtained MS spectra with the MS library MPW2007 [12] and by the retention time of the reference substance (tolperisone only). The tolperisone identification was also possible by HPLC–DAD as demonstrated in Fig. 2 for a whole blood extract. Tolperisone was identified by the retention time of the refrence substance and the obtained UV-spectra compared to that in the UV-spectra library of Pragst et al. [13]. Further qualitative results and the tolperisone concentrations in the different matrices are shown in Table 1.

The calculated whole blood tolperisone concentrations of 19 mg/l (case 1), 14 mg/l (case 2) and 7.0 mg/l (case 3) are much higher than the reported therapeutic blood concentrations [7,8]. The high concentrations of tolperisone in the gastric contents of all deceased indicate recent tolperisone ingestion. In contrast to frequently observed blood/plasma distribution ratios <1.0, the tolperisone post-mortem serum concentration of 12 mg/l in case 1 was lower than the corresponding whole blood concentration. The concentrations of paracetamol (case 1, 5.1 mg/l), ibuprofen (case 2, 14 mg/l) and naproxen (case 3, 48 mg/l) can be considered therapeutic. However, the influence of a potentially lethal paracetamol concentration in case 2 of 160 mg/l has to be taken into account, even though the circumstances of the death (time between last appearance and discovery of the deceased) suggested a rapid death occurred only a few hours after ingestion. Moreover,

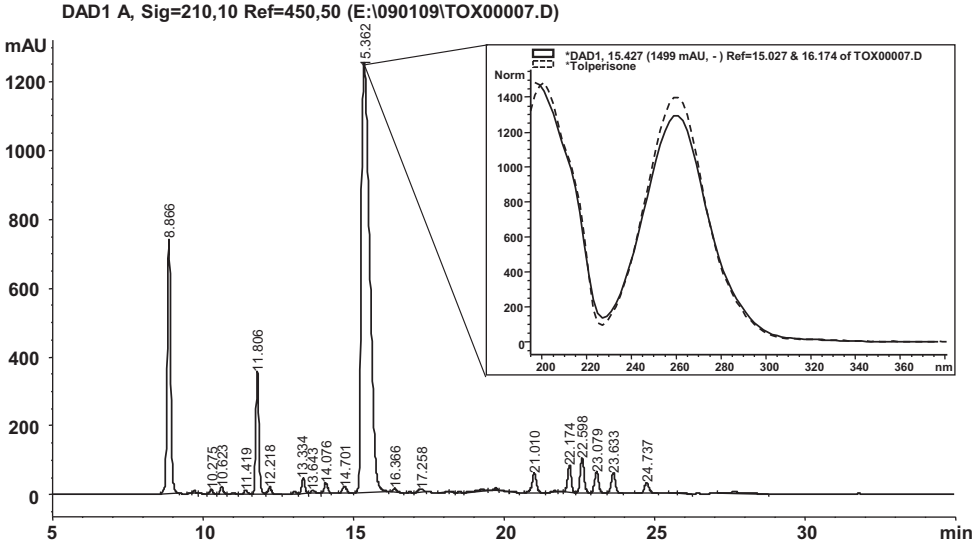

Fig. 2. HPLC chromatogram of a whole blood extract from case 3 with the UV-spectra and the corresponding library spectra for tolperisone [13].

Table 1
Qualitative and quantitative results of systematic toxicological analysis.

Case	Matrix	Tolperisone concentration	Other detected substances
1	Fluorinated femoral blood	19 mg/l	Paracetamol (5.1 mg/l)
	Urine	50 mg/l[a]	Hydroxy-tolperisone, paracetamol
	Gastric content	100 mg/40 ml[a]	Paracetamol
	Post-mortem serum	12 mg/l	
	Liver	8.7 mg/kg[a]	
	Bile	76 mg/l[a]	
	Cerebro-spinal fluid	9.5 mg/l[a]	
	Brain	4.9 mg/kg[a]	
2	Fluorinated femoral blood	14 mg/l	Paracetamol (160 mg/l), ibuprofen (14 mg/l)
	Urine	50 mg/l[a]	Hydroxy-tolperisone, paracetamol
	Gastric content	330 mg/105 g[a]	Paracetamol
3	Fluorinated femoral blood	7.0 mg/l	Naproxen (48 mg/l)
	Urine	11 mg/l[a]	Hydroxy-tolperisone, naproxen
	Gastric content	33 mg/90 g[a]	Naproxen

[a] estimated concentrations based on whole blood calibration.

histological findings did not show any sign of acute liver inflammation nor necrosis.

Because neither the macroscopic nor microscopic autopsy findings could explain the death in the three presented cases, acute tolperisone intoxication (case 1 and 3) and a mixed tolperisone/paracetamol intoxication (case 2) were interpreted as the most likely causes of death.

5. Conclusion

To the best of our knowledge, we report for the first time three cases involving the centrally acting muscle relaxant tolperisone (Mydocalm®) in fatal poisonings. Although tolperisone intoxications seem to occur rarely, the concentrations reported herein can help toxicologists interpret comparable incidents, especially when keeping in mind that two of the three cases can be considered fatalities due to tolperisone alone.

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