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CASE REPORT

TOXICOLOGY

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Fatal Intoxication Because of Trihexyphenidyl

ABSTRACT: Trihexyphenidyl (THP) is an anticholinergic agent with forensic toxicological interest. We present a case of a 59-year-old woman with a history of paranoid disorder, who was found dead in the house where she lived alone. The autopsy findings revealed no marked pathological changes. Toxicological analysis based on gas chromatography–mass spectrometry (GC–MS) analysis revealed THP and its major metabolite (hydroxy—THP) in blood and urine, with THP concentrations of 0.053 and 0.560 mg/L, respectively. The blood and urine ethanol concentrations were low 0.096 and 0.100 g/L, respectively. Based on these results, we determined the cause of death to be THP poisoning. It is suggested that rare case of death associated with THP overdosage should be taken in conjunction with central nervous system depressants (benzodiazepines, ethanol) and/or with other pathological disorders. Thus, our case could not be supportive for this allegation.

KEYWORDS: forensic science, forensic toxicology, trihexyphenidyl, poisoning, gas chromatography–mass spectrometry

Trihexyphenidyl (THP [benzhexol], Artane®; Hemofarm, Vršac, Serbia) is a potent anticholinergic drug used in the treatment of Parkinsonism and in the control of drug-induced extrapyramidal side effects (1,2). Its mode of action is preventing the effects of acetylcholine by blocking its binding to muscarinic cholinergic receptors at neuroeffector sites on smooth muscle, cardiac muscle, and gland cells in peripheral ganglia and in the central nervous system (CNS) (2). Side effects of THP include disturbance of recent memory, tachycardia, and bradycardia and can precipitate glaucoma in predisposed patients (3). Hydroxy-THP was reported as the major metabolite present in plasma and urine and accounted for two-thirds of the THP present in urine (4). Ethanol and other CNS depressants, such as anxiolytics, sedatives, and hypnotics, can increase the sedative effects of THP. The drug is of forensic toxicology interest because of its frequent abuse (5–8) and reported overdose (9), while fatal poisoning is rare (10,11).

The report describes a case of a woman found dead because of fatal poisoning involving THP. Autopsy results, toxicological findings, and analytical procedures are discussed.

Case Report

A 59-year-old woman with a history of paranoid disorder was found dead in the house where she lived alone. There were no suspicious circumstances, and a suicide note was not found. Later heteranamnestic data gained from her children revealed that she suffered from persistent delusional disorder (IDC-F22) and was being treated in an outpatient program. Her therapy was frequently changed, and each change was accompanied by her complaint of palpitations. The latest therapy approach included Moditen depo® (fluphenazine 25 mg injection, received 3 weeks before fatality; Krka, Novo Mesto, Slovenia) and Artane® (THP hydrochloride 15 mg/day). They last saw her 3 days prior the death.

Autopsy Report

The woman was 152 cm tall and weighed 55 kg. External examination of the body yielded no evidence of external injuries or violence. Autopsy findings revealed no marked pathological changes. Histologically, only vacuolar degeneration and cholestasis in liver were found (Fig. 1). Femoral venous blood, urine, bile, and gastric content were collected for toxicological analyses.

Toxicology Report

At the Institute of Forensic Medicine in Novi Sad, 95% of all autopsies in forensic medicine are routinely screened for ethanol and about 150 different substances, mainly pharmaceuticals. To reveal the presence of different drugs and/or metabolites in biological materials, samples are extracted separately from acidic (pH 2; 2M H_2SO_4 is added) and alkaline (pH 9; K_2CO_3:$NaHCO_3$ = 2:3 and 25% NH_4OH are added) media. Quantitative gas chromatography–mass spectrometry (GC–MS) analyses are performed after confirmation of the presence of acidic and/or basic drugs by qualitative GC–MS analysis (by matching both the retention time and full scan spectra of an unknown peak with a standard). In this case, the presence of THP and hydroxy-THP has been detected by screening method and the quantitative analysis was carried out by extraction method from alkaline media (described bellow).

Solution of the internal standard (IS) of meperidine (0.4 mL; 1.743 mg/L) was added to blood and gastric content samples (2 mL each), together with solid buffer K_2CO_3:$NaHCO_3$ = 2:3 and 25% NH_4OH to attain pH 9. To urine (5 mL) and bile (1 mL) samples, solution of IS of meperidine (0.4 mL; 1.743 mg/L) was added, and after acid hydrolysis, solid buffer K_2CO_3:$NaHCO_3$ = 2:3 and 25% NH_4OH was added to attain pH 9.

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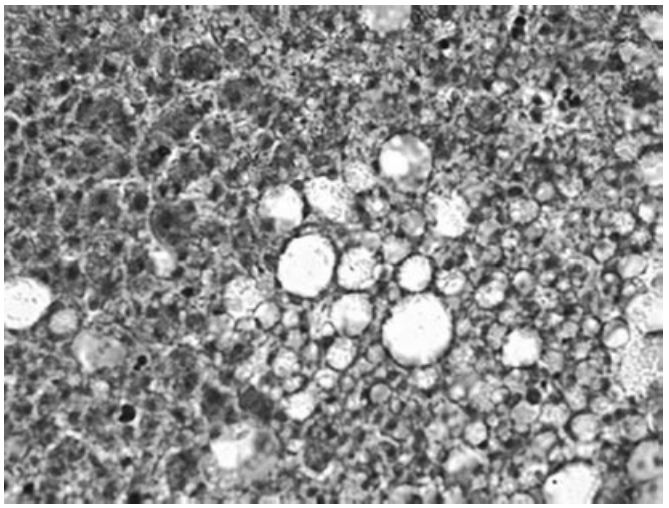

FIG. 1—*Section of the liver (×40) showing cholestasis and vacuolar degeneration.*

Blood, gastric content, and bile samples were extracted by solid phase extraction (SPE) using Extrelut NT 3 extraction columns (Merck, Darmstadt, Germany). Samples were loaded on columns, and after 20 min, analytes were evaluated with 15 mL of the mixture of solvents methylene chloride/methanol = 90:10. Urine sample was extracted by SPE using Extrelut NT 20 extraction column (Merck). Sample was loaded on column, and after 20 min, analytes were eluated with 2 × 20 mL of the mixture of solvents methylene chloride/methanol = 90:10. The eluates were evaporated to dryness under a gentle stream of nitrogen at 40°C, reconstituted in 0.4 mL of methylene chloride, and then transferred to autosampler vials for GC–MS analysis.

Qualitative and quantitative GC–MS analysis was performed using an Agilent 6890 N gas chromatograph (GC) equipped with Agilent 5973 mass selective detector, Agilent autosampler 7683, and Agilent DB–5MS capillary column (30 m, 0.25 i.d., 0.25 μm film thickness) (Agilent Technologies, Santa Clara, CA). The MS detector was operated in electron impact mode at 70 eV with interface temperature of 280°C. The injection port temperature was 250°C. GC was performed in splitless mode, and carrier gas was helium at a constant flow rate of 1 mL/min. The column temperature was programmed as follows: an initial temperature of 50°C increased to 130°C at a rate of 22°C/min, then to 280°C at a rate of 12°C/min, and was maintained for 12.86 min. Selected ion monitoring was carried out at *m/z* 98 and 218 for THP, and at *m/z* 247, 172, and 218 for IS of meperidine.

Separate stock solutions of THP and IS (meperidine) were prepared at concentration 100 mg/L in deionized water by diluting the original standard methanol solutions of THP (1 g/L) and meperidine (1 g/L), purchased from Sigma-Aldrich (Steinheim, Germany).

THP working solutions were prepared from stock solution up to final concentrations of: 0.02, 0.10, 0.50, 2.00, and 4.00 mg/L. IS working solution was prepared at the final concentration of 1.743 mg/L.

Calibration samples (spiked blood and urine samples) were prepared by adding aliquots of standard working solutions of THP to drug-free human blood (2 mL) and urine (5 mL) to final concentrations from 0.02 to 4.00 mg/L and 0.4 mL of standard working solutions of IS. All calibration samples were extracted according to the described method for the unknown samples.

Quantification was carried out on the basis of the characteristic *m/z* values of ions for THP. The ratio of the peak areas of THP and that of IS were presented as a function of the substance concentration using linear regression method, the coefficient of correlation being $r^2 = 0.998$. The lower detection limit for THP was 0.005 mg/L, and limit of quantitation was 0.015 mg/L. Recovery was determined by adding a known amount of THP to the control biological samples (drug-free human blood and urine), which were then treated on SPE columns, and after the GC–MS analysis, the peak areas of THP were compared with peak areas of the same amount of the THP injected directly to the GC–MS instrument without extraction. This way recovery achieved the range of 80–90%.

TABLE 1—*Trihexyphenidyl and ethanol concentrations in tissues.*

	Femoral Venous Blood	Urine	Bile
THP	0.053 mg/L	0.560 mg/L	ND
Hydroxy-THP	+	+	+
Ethanol	0.096 g/L	0.100 g/L	NA

+, the drug was detected, but not quantified; NA, not analyzed; ND, below lower detection limit (<0.005 mg/L); THP, trihexyphenidyl.

Ethanol was analyzed in femoral venous blood and urine by headspace GC with a flame ionization detector as described in detail previously (12).

The results from the toxicological analysis are given in Table 1. Qualitative GC–MS analysis confirmed the presence of THP in blood and urine, and hydroxy-THP in blood, urine, and bile. The presence of these substances and other xenobiotics was not confirmed in gastric content. Total ion chromatograms of blood and urine extract and full scan mass spectrum of THP from the blood extract are presented in Fig. 2*A–C*, respectively. The retention times of THP, hydroxy-THP, and meperidine were 16.05, 18.04, and 11.79 min, respectively. GC–MS quantitative analysis revealed THP concentration of 0.053 mg/L in femoral venous blood and 0.560 mg/L in urine. The blood and urine ethanol concentrations were 0.096 and 0.100 g/L, respectively.

Discussion

As a competitive antagonist of muscarinic receptors, THP is used in the treatment of parkinsonism and as adjuvant in control of side effects (extrapyramidal symptoms) during therapy with neuroleptics (especially phenothiazines). THP-hydrochloride is well absorbed from gastrointestinal tract producing average peak plasma levels at 1.3 h after single oral dose of 2 and 15 mg and reaching C_{max} of 0.01 and 0.05 mg/L, respectively (13). The half-life time varies from 3.6 up to 33 h, following multicompartmental kinetics (1,13). THP undergoes extensive metabolism, and hydroxy-THP was reported as the major metabolite (4). In adults, the therapeutic dose ranges from 1 to 20 mg daily (1,3). In some patients, THP doses of more than 12 mg daily may produce severe mental disturbance and excitement, while, on the other hand, doses up to 300 mg (5 mg/kg) have been ingested without fatalities or sequelae (10).

Death associated with the use of THP is a very rare occurrence. The following postmortem concentrations were observed in a 16-year-old youth who experienced hallucinations and was found dead in a nearby lake 2 days after the ingestion of 20 mg of THP: blood 0.03 mg/L and urine 0.38 mg/L (14). THP postmortem concentrations observed in a 48-year-old man with schizophrenia were 0.12 mg/L in femoral artery blood, 0.5 mg/kg in liver, and 0.4 mg in gastric content (10). Three other fatalities associated with THP have been reported, and in these cases, the blood concentrations have been in the range 0.03–0.24 mg/L (10). Other drugs were not detected. It was suggested that for fatalities to occur following

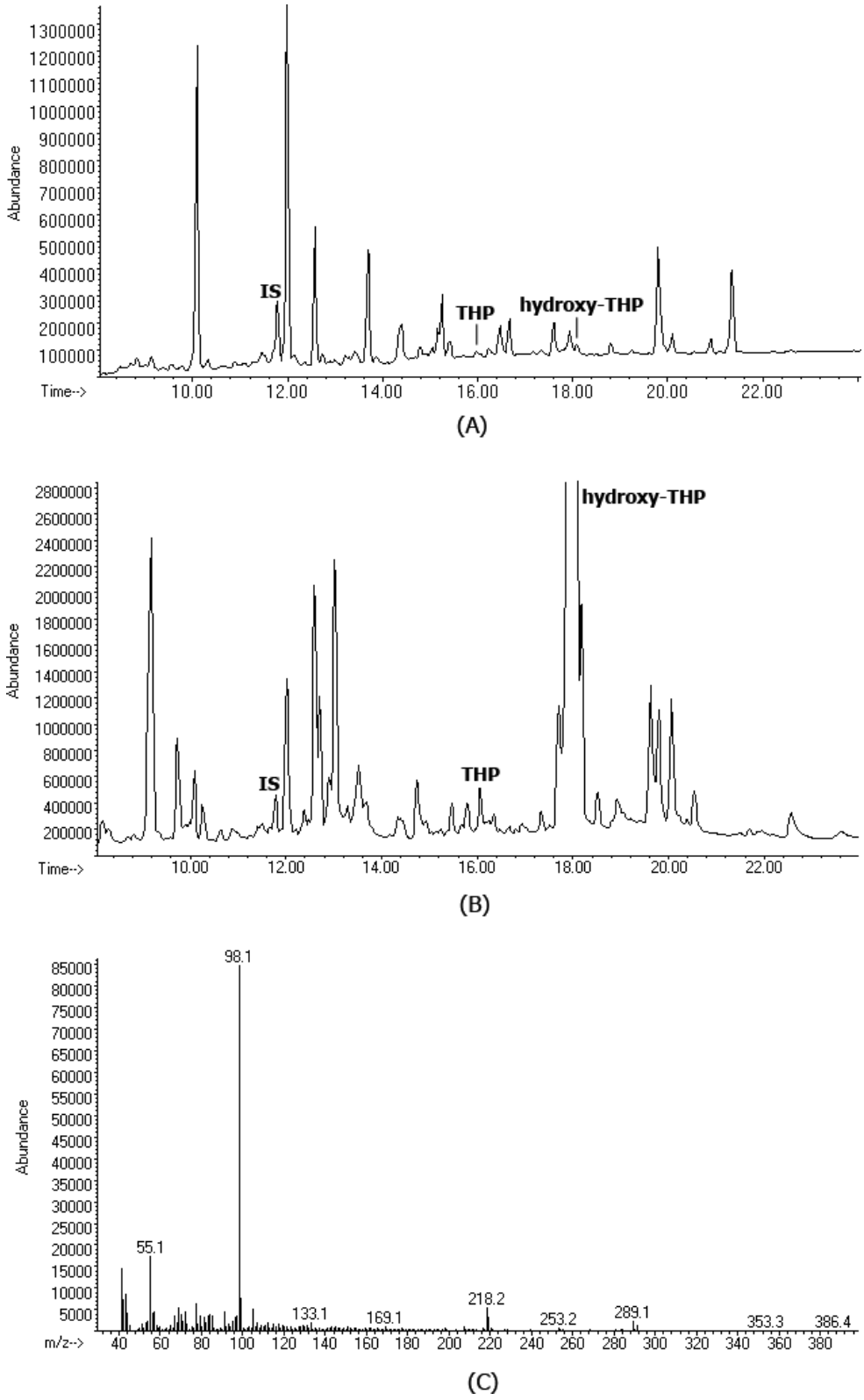

FIG. 2—*(A) TIC of blood extract, (B) TIC of urine extract, and (C) mass spectrum of THP from the blood extract. THP, trihexyphenidyl; TIC, total ion chromatograms.*

THP intoxication, secondary contributory factors, which probably further alter the patient's conscious state, are necessary. However, pathological picture in our case could not be supportive for this allegation.

However, rare cases of death associated with THP overdosages taken in conjunction with other CNS depressant agents have been reported too. Dowling and Robins (15) reported a fatality in a 55-year-old man with suicidal intent, following the consumption of an

uncertain quantity of amitriptyline and THP tablets. Toxicological analysis at autopsy revealed the blood amitriptyline, THP, and ethanol levels to be 0.27 mg/L, 0.8 mg/L, and 0.10 g/L, respectively. In a case where postmortem THP level was 0.24 mg/L, there was an associated significant concentration of both diazepam and ethanol together with a postmortem finding of aspiration of vomitus (10).

In our case, THP and its major metabolite (hydroxy-THP) were detected in blood and urine. The blood and urine ethanol concentrations were 0.096 and 0.100 g/L, respectively. THP blood and urine concentrations were found to be 0.053 and 0.560 mg/L, respectively, which are the concentrations associated with fatalities (10, 14).

The circumstances of the case exclude homicide. However, these data are not sufficient to determine neither suicide nor accident as a manner of death.

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