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An autopsy case of death by combined use of benzodiazepines and diphenidine

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SUMMARY

We present an autopsy case involving benzodiazepines and diphenidine. Quantitative toxicological analysis showed concentrations of 7-aminoflunitrazepam (a flunitrazepam metabolite), 7-aminonimetazepam (a nimetazepam metabolite), chlorpheniramine and diphenidine in femoral blood of 0.086 μg/ml, 0.027 μg/ml, 0.066 μg/ml, and 0.073 μg/ml, respectively. Death was attributed to combined toxicity due to the influence of multiple drug interactions.

Keywords: multiple drug interaction – benzodiazepine – diphenidine

Smrteľný prípad kombinovaného užitia benzodiazepínov a difenidínu.

SÚHRN

Pitva prípadu smrtelnej otravy kombináciou benzodiazepínov a diphenidinu.
V súdnolekárskej praxi sa stretávame pomerne často s požitím liečiv a psychotropných a omamných látok.. V práci uvádzame prípad smrti v dôsledku kombinovaného užitia benzodiazepínov a difenidínu ako novou psychoaktívnou látkou.
Dvadsať ročná žena bola nájdená v jej izbe., pričom v jej blízkosti boli prítomné početné prázdne balíčky liekov na predpis a balík difenidínu s príslušenstvom na fajčenie. Pri pitve neboli pozorované žiadne významné nálezy, ktoré by mohli inak súvisieť s príčinou úmrtia. Za účelom detekcie významných cudzorodých látok boli vykonané toxikologické analýzy pomocou plynovej chromatografie s hmotnosto spektrometrickým detektorom (GC-MS) a kvapalinovej chromatografie s hmotnosto spektrometrickým detektorom (LC / QTOF-MS). Kvantitatívna toxikologická analýza krvi odobratej zo stehnovej žily ukázala, že boli prítomné látky ako 7-aminoflunitrazepam (metabolit flunitrazepamu), 7-aminonimetazepam (metabolit nimetazepamu), chlorfeniramín a difenidín v koncetráciách 0.086 μg/ml, 0.027 μg/ml, 0.066 μg/ml a 0.073 μg/ml v uvedenom poradí. Smrť bola dôsledkom kombinovanej otravy viacerými liečivami a difenidínom, novodobou dizajnérskou drogou.
Na základe pitevného nálezu zomrelej, výsledkov toxikologického vyšetrenia a vyšetrovania zo strany úradov sme dospeli k záveru, že užitie liekov z radu benzodiazepínov, antagonistov histamínového receptora H1 a dizajnérskej drogy difenidínu viedlo k jej smrti v dôsledku ich kombinovanej toxicity. V literatúre sú nedostatočné údaje týkajúce sa interakcie liekov v prípadoch viacpočetného užívania drog, nami uvedená štúdia poukazuje aj na to, že by sa mala venovať väčšia pozornosť takýmto kombinovaným otravám.

Kľúčové slová: kombinovaná intoxikácia – benzodiazepíny – difenidín.

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Ingestion of multiple psychotropic drugs is sometimes observed in forensic cases (1-3). Flunitrazepam and nimetazepam are benzodiazepine derivatives, both widely used as hypnotics (4,5). Diphenidine, a new psychoactive substance, appeared on the illegal market at the end of 2013 in Japan (6).

We report herein a case of death due to combined use of multiple psychotropic drugs with diphenidine, and discuss the interactions among these drugs.

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CASE REPORT

A female in her twenties was found dead lying face down on her bed. Numerous empty packets of prescription drugs and a package of new psychoactive substances with an implement for smoking were nearby. Subsequent police investigations revealed that the deceased had been prescribed medication for insomnia. Medico-legal autopsy revealed slight abrasions on the dorsal surface of the both hands, but these were not considered contributory to the cause of death. No findings of natural disease were observed.

The deceased was 157 cm in height and weighed 45 kg. Her heart weighed 220 g and contained 140 ml of blood without coagulum. The brain weighed 1413 g and was slightly edematous. The left and right lungs weighed 389 g and 460 g, respectively, and were congested. Histological examination revealed marked congestion and edema in the lungs. No notable changes were evident in the other organs, other than congestion.

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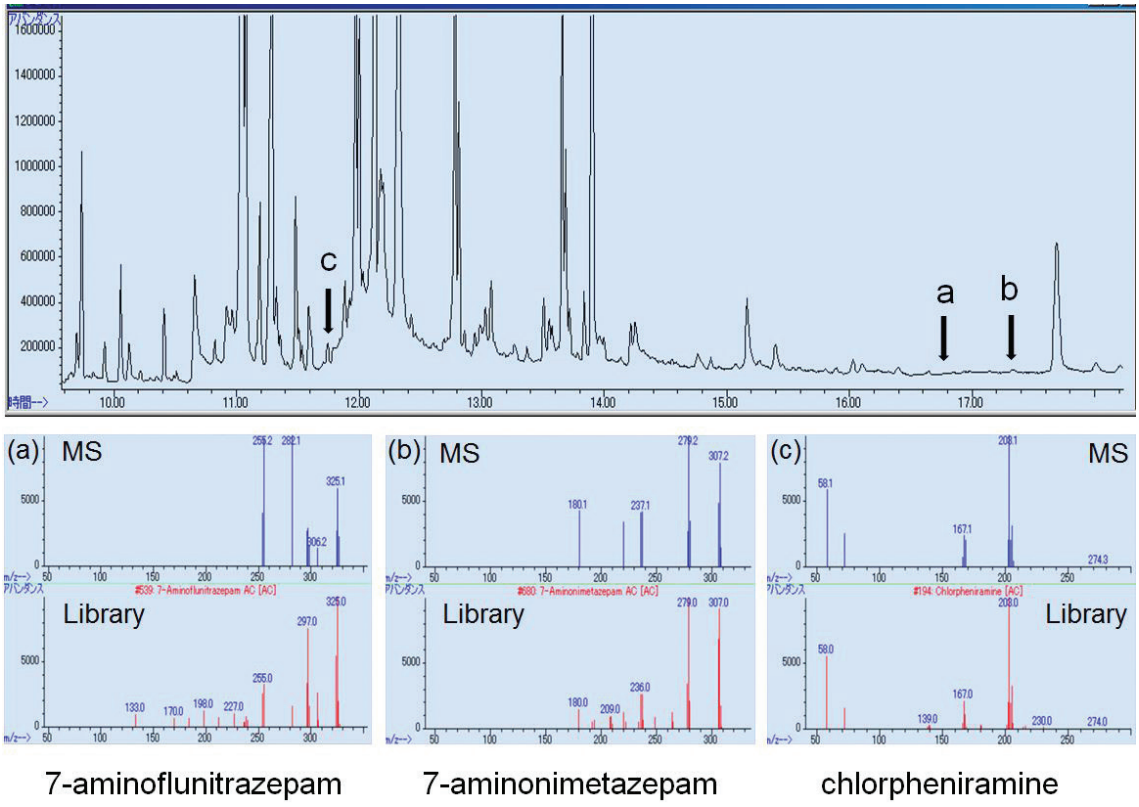

Figure 1. Total ion chromatogram (TIC) and mass spectrum of (**a**) 7-aminoflunitrazepam, (**b**) 7-aminonimetazepam and (**c**) chlorpheniramine in blood sample.

A drug screening test using a Triage™ panel (Biosite Diagnostic, San Diego, CA) yielded positive results for benzodiazepines. Postmortem blood samples were collected for toxicological investigation.

TOXICOLOGICAL EXAMINATION

Toxicological analysis using gas chromatography–mass spectrometry (GC-MS) was performed with a slight modificati-

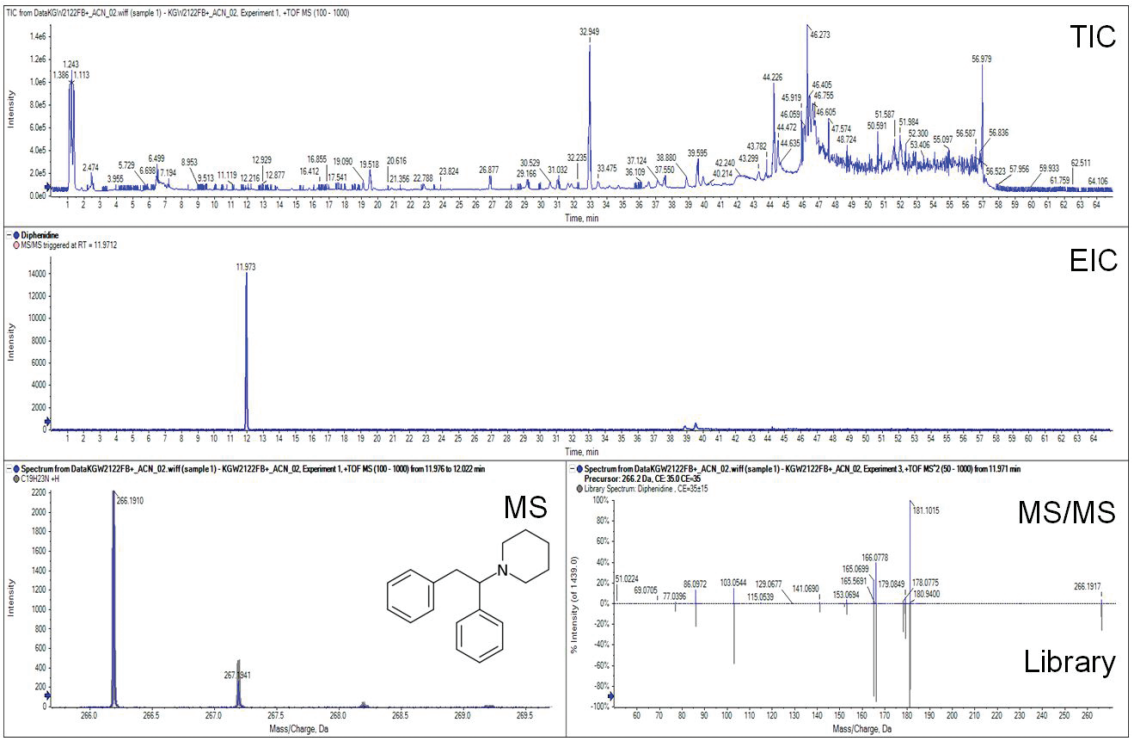

Figure 2. Total ion chromatogram (TIC), extracted ion chromatogram (EIC) and mass spectrum of diphenidine in blood sample.

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Table 1. Concentrations found for each drug and metabolite in the post-mortem blood (μg/ml).

Specimen	Blood concentration	Therapeuticl range *	Toxic range	Lethal range *
flunitrazepam	B.D.L	0.005-0.015	0.01-0.05	>0.16 **
7-aminoflunitrazepam	0,086			
nimetazepam	B.D.L	0.013 †	No data	No data
7-aminonimetazepam	0,027	No data	No data	No data
nitrazepam	B.D.L	0.03-0.1	0.2-3	5
chlorpheniramine	0,066	0.003-0.017	-	1.18
diphenidine	0,073	-	No data	>0.71 ¶

** Therapeutic and lethal ranges are cited from the reference (8).*
*** Lethal range includes total of flunitrazepam and 7-aminoflunitrazepam (9).*
† Cmax data from interview form (10).
¶ The data is cited from the reference (11).
B.D.L: below the detection limit

on of a previously reported method (7). In brief, a GC-MS, 6890N gas chromatograph combined with a 5973MS mass spectrometer (both Agilent Technologies, Santa Clara, CA) was used. The gas chromatograph was fitted with a DB-5MS column (30m × 0.25 mm I.D., film thickness, 0.25μm; Agilent Technologies). Identification of each compound was determined by retention times and confirmation ions (7-aminoflunitrazepam, 7-aminonimetazepam and chlorpheniramine for m/z=325, 279 and 203, respectively). The blood samples were extracted with the Focus® columns (Agilent Technologies) and incubated with the acetylating reagents according to the pervious report (7). We have used diazepam-d_5 (Hayashi Pure Chemical Ind Ltd., Osaka, Japan) as internal standard, and the recovery of each compound at the level of 1μg/ml was obtained by comparing the peak area of each compound in a whole blood extract with that in the standard solution.

Determination and quantification of diphenidine was performed by liquid chromatography quadrupole time-of-flight-mass spectrometry (LC/QTOF-MS). In brief, liquid chromatography separations were carried out using Nexera (Shimadzu, Kyoto, Japan). A ZORBAX Eclipse Plus C18 (2.1 mm × 100 mm, 1.8-μm particle size; Agilent, Santa Clara, CA) was used with a mobile phase of solvent A (0.1% formic acid containing 10 mM ammonium formate) and solvent B (acetonitrile). A Triple TOF® 5600 mass spectrometer (AB Sciex, Framingham, MA) was used to obtain accurate mass and MS/MS spectra. Quantitation of ethanol was performed using headspace gas chromatography.

RESULTS AND DISCUSSION

Toxicological analysis identified 7-aminoflunitrazepam (metabolite of flunitrazepam), 7-aminonimetazepam (metabolite of nimetazepam), chlorpheniramine (Figure 1) and diphenidine (Figure 2), respectively. Neither nitrazepam (as a metabolite of nimetazepam) nor ethanol was detected in the blood. Table 1 shows the quantification for each drug in the blood, along with the currently established therapeutic, toxic and fatal levels (8-11). Analytical results indicated the presence of a toxic level of 7-aminoflunitrazepam, while chlorpheniramine was slightly over the normal therapeutic range (0.003-0.017 μg/ml) (8). Diphenidine was at about one-tenth of the fatal range, and data are currently lacking regarding the toxic range for 7-aminonimetazepam.

Diphenidine is a *N*-methyl-D-aspartic acid (NMDA) receptor antagonist (11-13). NMDA antagonists exert protective effects against neuronal death and dissociative anesthetic effects (14), and diphenidine alters the level of consciousness (12). Details of the pharmacological effects and toxicity are not well known, but additive effects may result when combined with other depressants such as benzodiazepines (15). In the present case, one-tenth of a fatal level of diphenidine was detected.

Among the detected drugs, 7-aminoflunitrazepam and 7-aminonimetazepam are metabolites of flunitrazepam and nimetazepam, respectively (4,16-18). Both 7-aminoflunitrazepam and 7-aminonimetazepam in blood are markers of the intake of the parent drugs flunitrazepam and nimetazepam, respectively. As both flunitrazepam and nimetazepam are derivatives of nitrobenzodiazepine and nitrazepam (4,5), they have similar chemical structures and have some ranges of those therapeutic levels (4,5,18). Although no information is currently available on the toxicity of nimetazepam, toxicity and pharmacological actions were considered likely to resemble those of flunitrazepam, and blood levels seemed to be in the toxic range. Chlorpheniramine is a first-generation histamine H_1 receptor antagonist that is used to treat symptoms of the common cold and rhinorrhea (4). The postmortem blood concentration of chlorpheniramine in the present case was above the therapeutic range, but seems to be below the fatal range (8). As histamine acts as a neurotransmitter in the central nervous system (CNS), first-generation H_1 receptor antagonists such as chlorpheniramine cause sedation, even in conventional therapeutic doses (19). Such drugs also reinforce the pharmacological action of CNS depression induced by benzodiazepines (9).

We have calculated the victim's total amounts of intake of flunitrazepam and chlorpheniramine, using values of the distribution volume (Vd) for flunitrazepam (3.4-5.5L/kg) and chlorpheniramine (3-6L/kg) (4), the victim's body weight and each blood levels. We therefore estimated that she had ingested at least 13.1 mg of flunitrazepam and 8.9 mg of chlorpheniramine, respectively. However, as we could not obtain the values of Vd for both nimetazepam and diphenidine, we could not estimate total intake of those drugs.

Based on the autopsy findings, the results of toxicological examination and the investigation by the authorities, we concluded that multiple drug ingestion including benzodiazepines, histamine H_1 receptor antagonist and new psychoactive substances, led to her death due to their combined toxicity. Although scant data exists concerning drug interaction effects in cases of multiple drug use, the present case shows that more attention should be paid to multiple psychotropic drugs.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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ZPRÁVA ZE SJEZDU

6. trilaterální sympozium, Rožnov pod Radhoštěm

Ve dnech 24. – 26. 5. 2017 se uskutečnilo již 6. Trilaterální sympozium s mezinárodní účastí v Rožnově pod Radhoštěm. Přednáškový program se tradičně konal v Janíkově stodole Valašského muzea v přírodě a v přednáškových prostorách Beskydského hotelu Relax, ve spolupráci úrazové chirurgie, traumatologie, Policie ČR a soudního lékařství. Sympozium slavnostně zahájil prezident kongresu doc. MUDr. Leopold Pleva, CSc., který byl oficiálně oceněn policejním prezidentem genmjr. Mgr. Bc. Tomášem Tuhým. Při slavnostním zahájení byl přítomen též jeden z hlavních organizátorů MUDr. Igor Dvořáček, Ph.D. Následoval dvoudenní odborný program, během kterého zaznělo celkem 24 ústních sdělení českých, slovenských a polských kolegů z řady medicínských i jiných oborů a byla k dispozici i posterová sekce. Účastníci sympozia byli po čtvrtečním odborném programu odměněni vepřovými hody v prostorách skanzenu Valašského muzea a následně společenským programem v Beskydském hotelu Relax. Celkem 225 registrovaných posluchačů z řad odborné veřejnosti tak mělo bohatý vzdělávací i kulturní program.

Krásné prostory rožnovského Valašského muzea v přírodě opět za dva roky přivítají již 7. Trilaterální sympozium.

Text: Michaela Ublová, Miroslav Šafr
Foto: Igor Dvořáček