CASE REPORTS

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Fatal poisoning case involving drug interaction due to the inhibition of cytochrome P450

Slučaj trovanja sa fatalnim ishodom koji uključuje interakciju lekova zbog inhibicije citohroma P450

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Abstract

Introduction. Cytochrome P450 (CYP) enzymes are responsible for the metabolism of various drugs and chemicals. The forensic pathologist should consider not only pharmacodynamic interactions by multiple drugs but also the pharmacokinetic drug interactions of each drug in the case of multiple drug ingestion. **Case report.** A female in her forties, receiving therapy for alcohol dependence, was found dead in her house. The medicolegal autopsy revealed no findings suggestive of natural cause of death. Quantitative toxicological analysis showed that levomepromazine, promethazine, dextromethorphan, estazolam, clomipramine, risperidone, and flunitrazepam concentrations in femoral blood were 0.750 μg/mL, 0.701 μg/mL, 0.332 μg/mL, 0.390 μg/mL, 0.216 μg/mL, 0.031 μg/mL, and 0.002 μg/mL, respectively, along with a blood ethanol level of 377 mg/dL. **Conclusion.** We concluded that the cause of death was the interaction between ethanol and multiple psychotropic drugs. Pharmacokinetic drug interactions due to the CYPs inhibition should be considered in the evaluation of toxicity in poisoning cases involving multiple drugs.

Key words:
autopsy; cytochrome p-450 enzyme system; drug interactions; ethanol; forensic pathology; phenothiazines; poisoning; psychotropic drugs.

Apstrakt

Uvod. Enzimi citohroma P450 (CYP) su odgovorni za metabolizam različitih lekova i hemikalija. Forenzički patolog treba da razmotri ne samo farmakodinamske interakcije između više lekova već i farmakokinetičke interakcije svakog leka u slučaju multiple ingestije lekova. **Prikaz bolesnika.** Žena u 40-im godinama, na lečenju od zavisnosti od alkohola, nađena je mrtva u svojoj kući. Medikolegalnom autopsijom nisu nađeni znaci koji bi upućivali na prirodni uzrok smrti. Kvantitativnom toksikološkom analizom nađeno je da su koncentracije levomepromazina, prometazina, dekstrometorfana, estazolama, klomipramina, risperidona i flunitrazepama u femoralnoj krvi bile: 0.750 μg/mL, 0.701 μg/mL, 0.332 μg/mL, 0.390 μg/mL, 0.216 μg/mL, 0.031 μg/mL, i 0.002 μg/mL, redom, uz etil alkohol u krvi u koncentraciji od 377 mg/dL. **Zaključak.** Zaključili smo da je uzrok smrti bila interakcija između etil alkohola i više psihotropnih lekova. Farmakokinetičke interakcije lekova zbog inhibicije CYP trebalo bi uzeti u obzir kod procene toksičnosti u slučajevima trovanja koji uključuju ingestiju više lekova.

Ključne reči:
autopsija; citohrom p-450; lekovi, interakcija; alkohol, etil; patologija, sudska; fenotiazini; trovanje; psihotropni lekovi.

Introduction

Fatal cases following multiple drug ingestion are sometimes observed in forensic casework. In such cases, the forensic pathologist should consider not only pharmacodynamic interactions between multiple drugs but also the pharmacokinetic drug interactions of each drug [1]. Cytochrome P450 (CYP) enzymes are responsible for the metabolism of various drugs and chemicals, including psychotropic drugs [2]. CYP3A4 and CYP2D6 are two major enzymes involved in drug metabolism [2, 3]. These two enzymes metabolize approximately 50% of drugs [2]. CYP2D6 is involved in the metabolism of many psychotropic drugs, such as antipsychotic agents, tricyclic antidepressants, opioids, and antiarrhythmic agents [4, 5]. Hence, inhibition of these enzymes results in significant pharmacokinetic drug interactions, which can cause

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adverse reactions. Here, we report a case of death due to the combined use of ethanol with multiple psychotropic drugs and discuss the pharmacokinetic interactions among them.

Case report

A female in her forties was found dead in her house. Subsequent police investigations revealed that the deceased was receiving therapy for alcohol dependence. Although she had been prescribed psychotropic drugs, such as estazolam, flunitrazepam, levomepromazine, promethazine, and risperidone, she could not stop drinking. The medicolegal autopsy revealed no injury except an old scar on her forehand. No findings suggestive of natural disease and, consequently, natural cause of death was not observed.

The deceased was 161 cm tall and had 84.5 kg. Her heart weighed 386 g and contained 200 mL of blood without coagulum. Her brain weighed 1,470 g and was slightly edematous. The left and right lung weighed 475 g and 642 g, respectively, and were congested. The stomach contained foodstuff in greyish fluid (100 mL). There were no notable changes in other organs other than congestion. A drug screening test using a Triage™ (Biosite Diagnostic Inc., San Diego, CA, USA) panel was positive for benzodiazepines. *Postmortem* blood, urine, bile, and stomach content samples were collected for toxicological investigation.

Toxicological analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed using a slightly modified method from that previously reported [6]. In brief, the liquid chromatography separations were carried out using Ekspert™ UltraLC 100-XL (Eksigent part of AB Sciex, Framingham, MA, USA). An L-column2 ODS (1.5 mm × 150 mm, 5.0 μm particle size, Chemicals Evaluation and Research Institute, Tokyo, Japan) was used with a mobile phase of solvent A (5% methanol containing 10 mM ammonium formate) and solvent B (95% methanol containing 10 mM ammonium formate) with a flow rate of 0.1 mL/min. A QTrap® 4500 tandem mass spectrometer (AB Sciex) was used to obtain the mass spectra. Quantitation of ethanol was performed using headspace gas chromatography.

The toxicological analysis identified levomepromazine, promethazine, dextromethorphan, estazolam, clomipramine (and its metabolite desmethylclomipramine), risperidone (and its metabolite paliperidone), flunitrazepam (and its metabolite 7-aminoflunitrazepam), and acetaminophen in the subject's body fluid samples. Ethanol concentrations in blood and urine were 377 mg/dL and 406 mg/dL, respectively. Table 1 shows the quantification of each drug in the victim's blood, along with the currently established fatal and therapeutic levels [7–9].

Table 2 shows the metabolic enzymes for each drug and the enzymes inhibited by each drug found in the patient's blood.

Table 1

Concentrations of each drug and metabolite in the *postmortem* samples (μg/mL)

Drug	Blood	Urine	Bile	Stomach contents	Therapeutic range *	Toxic range *	Lethal range *
Levomepromazine	0.750	0.056	2.206	41.493	0.005–0.2	0.4	0.5
Promethazine	0.701	0.062	1.501	15.847	0.05–0.4	1–2	1.8–5.4
Dextromethorphan	0.332	0.333	1.097	2.266	0.01–0.04	0.1	3
Estazolam	0.390	0.100	2.457	2.354	0.055–0.2	–	0.48
Clomipramine	0.216	0.023	1.912	1.928	0.02–0.4	0.4–0.6	1–2
Desmethylclomipramine	0.005	0.001	0.076	0.063	–	–	–
Risperidone	0.031	0.058	0.206	0.780	0.002–0.02	0.12	1.8
Paliperidone	0.015	0.057	0.470	0.149	0.02–0.06	0.12	–
Flunitrazepam	0.002	0.001	B.D.L	0.291	0.005–0.015	0.05	> 0.16 **
7–aminoflunitrazepam	0.011	0.002	0.541	0.131	–	–	
Acetaminophen	0.065	7.491	12.203	B.D.L	5–25	100–150	200–300

*** Therapeutic, toxic, and lethal ranges are cited [7, 8]; ** Lethal range includes the total of flunitrazepam and 7-aminoflunitrazepam [9]; B.D.L – below the detection limit.**

Table 2

Enzymes involved in the metabolism of each drug and enzyme inhibition by each drug

Drug	Metabolic enzyme	Enzyme inhibition
Levomepromazine	CYP3A4 [12]	CYP1A2, CYP2D6, CYP3A4 [5, 12]
Promethazine	CYP2D6 [5]	CYP2D6 [5, 13]
Dextromethorphan	CYP2D6, CYP3A4 [4, 15]	
Estazolam	CYP3A4 [16]	
Clomipramine	CYP1A2, CYP2C19, CYP2D6 [4, 5, 13]	CYP2D6 [4, 5, 13]
Risperidone	CYP2D6, CYP3A4 [2, 5]	
Flunitrazepam	CYP2C19, CYP3A4 [17]	
Acetaminophen	CYP1A2, CYP2E1 [5]	

Discussion

Analytical results indicated a fatal level of levomepromazine and toxic levels of promethazine, dextromethorphan, and estazolam in blood, while risperidone (and its metabolite paliperidone) and clomipramine (and its metabolite desmethylclomipramine) were in their therapeutic ranges, and flunitrazepam (and its metabolite 7-

aminoflunitrazepam) and acetaminophen were below their therapeutic ranges.

Levomepromazine is a phenothiazine derivative used as an antipsychotic drug and has pronounced sedative effects. Its overdose results in nausea, vomiting, coma, and convulsions [8]. Promethazine is also a phenothiazine derivative that is used as an antihistaminic, antiemetic, and sedative agent, and can cause coma, respiratory depression, and circulatory failure in overdose [8]. These drugs, along with ethanol, exert additive or synergistic effects, resulting in suppression of central nervous system function [10]. Based on autopsy findings and the results of the toxicological examination, we concluded that the combined toxicity of ethanol and multiple psychotropic drugs led to death in this case.

Most of the drugs are metabolized by CYPs [2, 4, 5, 10–17]. However, many of these drugs also inhibit CYPs. Among them, levomepromazine, promethazine, and clomipramine have potent inhibitory effects on CYP2D6, and levomepromazine also inhibits CYP3A4 [5, 11–13].

CYP2D6 is part of a group of enzymes responsible for the metabolism of many drugs. Since more than 65 commonly used drugs are metabolized by this enzyme [4], considering pharmacokinetic drug interactions in cases of multiple drug ingestion is important. The subfamily of CYP3A (including CYP3A4) is probably the most important of all drug-metabolizing enzymes [4].

Individual differences in the activity of CYP2D6 due to genetic polymorphism have been reported, based on which individuals are categorized as the following four phenotypes: poor, intermediate, extensive, and ultrarapid metabolizers [2, 4, 15]. Dextromethorphan is a synthetic analogue of codeine, which is prescribed as an antitussive agent. It is also used as a clinical probe for CYP2D6 phenotyping, as its major metabolic pathway is mediated by both CYP2D6 and CYP3A4 [8, 15]. The postmortem blood concentration of dextromethorphan in the present case was within the toxic range. Life-threatening intoxication with dextromethorphan resulting from inhibition of CYP2D6 as a result of interactions of other drugs has been clinically reported [18, 19]. Based on previous evidence, it is speculated that the drug levels in the present case might have been elevated due to the inhibition of CYP2D6 by drugs such as levomepromazine, promethazine, and clomipramine, and of CYP3A4 and CYP1A2 by levomepromazine. The inhibitory effects of other drugs depend on their dose and the ability of the inhibitor to bind to the enzyme, which usually occurs immediately after drug ingestion [3]. Estazolam is a triazolobenzodiazepine derivative metabolized by CYP3A4, and is prescribed for the short-term management of insomnia; somnolence, respiratory depression, and coma are observed with its overdose [8, 16]. In the current case, blood estazolam level might also have been elevated due to the inhibition of CYP3A4.

We speculate that the accumulation of dextromethorphan and estazolam in the patient's blood was possibly due to an inhibitory pharmacokinetic drug interaction. Thus, these two drugs might also have contributed to the patient's death.

The present case showed that more attention should be paid to the interactions between multiple psychotropic drugs.

Conclusion

We concluded that the cause of the patient's death was the interaction between ethanol and multiple psychotropic drugs. Pharmacokinetic drug interactions due to the inhibition of CYPs should be considered in the evaluation of toxicity.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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