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Authors Hiroshi Kinoshita, Naoko Tanaka, Mitsuru Kumihashi, Mostofa Jamal, Asuka Ito, Tadayoshi Yamashita, Kiyoshi Ameno, Vojnosanitetski pregled (2019); Online First September, 2019.

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FATAL POISONING CASE INVOLVING DRUG INTERACTION DUE TO THE
INHIBITION OF CYTOCHROME P450

Hiroshi Kinoshita, Naoko Tanaka, Mitsuru Kumihashi, Mostofa Jamal, Asuka Ito,
Tadayoshi Yamashita, Kiyoshi Ameno

Departments of Forensic Medicine, Faculty of Medicine, Kagawa University, 1750-1 Miki,
Kita, Kagawa 761-0793, Japan

Correspondence to: Hiroshi Kinoshita,
Department of Forensic Medicine, Faculty of Medicine, Kagawa University
1750-1 Miki, Kita, Kagawa 761-0793, Japan
Phone: +81 87 891 2140.
E-mail: kinochin@med.kagawa-u.ac.jp
Abstract

**Background/Aim.** Cytochrome P450 (CYP) enzymes are responsible for the metabolism of various drugs and chemicals. Forensic pathologist should consider not only pharmacodynamic interactions by multiple drugs, but also the pharmacokinetic drug interactions of each drug in casework of multiple drug ingestion. **Case report.** A female in her forties, receiving therapy for alcohol dependence, was found dead in her house. Medico-legal autopsy revealed no findings suggestive of natural disease. 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 interaction between ethanol and multiple psychotropic drugs. Pharmacokinetic drug interactions due to the inhibition of CYPs should be considered in the evaluation of toxicity in poisoning cases involving multiple drugs.

**Key words:**

drug interaction; overdose; levomepromazine; cytochrome P450 (CYP); dextromethorphan.

Apstrakt

**Uvod.** Enzimi P450 (CYP) su odgovorni za metabolizam različitih lekova i hemikalija. Forenzički patolog treba da razmotri ne samo farmakodinamske interakcije više lekova već i farmakokinetičke interakcije svakog leka u slučaju multiple ingestije lekova. **Prikaz bolesnika.** Žena 40-ih godina, na lečenju od zavisnosti od alkohola, nađena je mrtva u svojoj kući. Medikolegalnom autopsijom nisu nađeni zanci koji bi upućivali na prirodnu bolest. 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, and 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-a treba da budu razmotrene u proceni toksičnosti kod slučajeva trovanja koji uključuju više lekova.

**Ključne reči:**
interakcija lekova, overdoza, levomepromazin, citohrom P450 (CYP), dekstrometorfan.

**Introduction**

Fatal cases following multiple drug ingestion are sometimes observed in forensic caseworks. 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 adverse reactions. Here, we report a case of death due to combined use of ethanol with multiple psychotropic drugs, and discuss the pharmacokinetic interactions among these drugs.

**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. Medico-legal autopsy revealed no injury except an old scar on her forehead. No findings suggestive of natural disease were observed.

The deceased was 161 cm in height and 84.5 kg in weight. 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 lungs weighed 475 g and 642 g, respectively, and were congested. The stomach contained foodstuff in greyish fluid (100 mL). There were no notable changes, other than congestion, in the other organs. 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. 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.
Results and Discussion

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 1. Concentrations of each drug and metabolite in the post-mortem samples (µg/mL).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Blood</th>
<th>Urine</th>
<th>Bile</th>
<th>Stomach contents</th>
<th>Therapeutic range *</th>
<th>Toxic range *</th>
<th>Lethal range *</th>
</tr>
</thead>
<tbody>
<tr>
<td>levomepromazine</td>
<td>0.750</td>
<td>0.056</td>
<td>2.206</td>
<td>41.493</td>
<td>0.005-0.2</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>promethazine</td>
<td>0.701</td>
<td>0.062</td>
<td>1.501</td>
<td>15.847</td>
<td>0.05-0.4</td>
<td>1-2</td>
<td>1.8-5.4</td>
</tr>
<tr>
<td>dextromethorphan</td>
<td>0.332</td>
<td>0.333</td>
<td>1.097</td>
<td>2.266</td>
<td>0.01-0.04</td>
<td>0.1</td>
<td>3</td>
</tr>
<tr>
<td>estazolam</td>
<td>0.390</td>
<td>0.100</td>
<td>2.457</td>
<td>2.354</td>
<td>0.055-0.2</td>
<td>-</td>
<td>0.48</td>
</tr>
<tr>
<td>clomipramine</td>
<td>0.216</td>
<td>0.023</td>
<td>1.912</td>
<td>1.928</td>
<td>0.02-0.4</td>
<td>0.4-0.6</td>
<td>1-2</td>
</tr>
<tr>
<td>desmethylclomipramine</td>
<td>0.005</td>
<td>0.001</td>
<td>0.076</td>
<td>0.063</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>risperidone</td>
<td>0.031</td>
<td>0.058</td>
<td>0.206</td>
<td>0.780</td>
<td>0.002-0.02</td>
<td>0.12</td>
<td>1.8</td>
</tr>
<tr>
<td>paliperidone</td>
<td>0.015</td>
<td>0.057</td>
<td>0.470</td>
<td>0.149</td>
<td>0.02-0.06</td>
<td>0.12</td>
<td>-</td>
</tr>
<tr>
<td>flunitrazepam</td>
<td>0.002</td>
<td>0.001</td>
<td>B.D.L</td>
<td>0.291</td>
<td>0.005-0.015</td>
<td>0.05</td>
<td>&gt;0.16</td>
</tr>
<tr>
<td>7-aminoflunitrazepam</td>
<td>0.011</td>
<td>0.002</td>
<td>0.541</td>
<td>0.131</td>
<td>-</td>
<td>-</td>
<td>**</td>
</tr>
<tr>
<td>acetaminophen</td>
<td>0.065</td>
<td>7.491</td>
<td>12.203</td>
<td>B.D.L</td>
<td>5-25</td>
<td>100-300</td>
<td>200-300</td>
</tr>
</tbody>
</table>

* Therapeutic, toxic and lethal ranges are cited from the reference [7,8].

** Lethal range includes total of flunitrazepam and 7-aminoflunitrazepam [9].

B.D.L: below the detection limit
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 that is used as an antipsychotic drug and has pronounced sedative effects. Its overdose results in nausea, vomiting, coma, and convulsions. 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. These drugs, along with ethanol, exert additive or synergistic effects, resulting in suppression of central nervous system function. Based on autopsy findings and the results of toxicological examination, we concluded that combined toxicity of ethanol and multiple psychotropic drugs led to death in this case.

Most of the drugs are metabolized by CYPs. 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. Table 2 shows the metabolic enzymes for each drug and the enzymes inhibited by each drug found in the present case’s blood.

<table>
<thead>
<tr>
<th>drug</th>
<th>metabolic enzyme</th>
<th>enzyme inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>levomepromazine</td>
<td>CYP3A4 [12]</td>
<td>CYP1A2, CYP2D6, CYP3A4 [5,12]</td>
</tr>
<tr>
<td>promethazine</td>
<td>CYP2D6 [5]</td>
<td>CYP2D6 [5,13]</td>
</tr>
<tr>
<td>dextromethorphan</td>
<td>CYP2D6, CYP3A4 [4,15]</td>
<td></td>
</tr>
<tr>
<td>estazolam</td>
<td>CYP3A4 [16]</td>
<td></td>
</tr>
<tr>
<td>clomipramine</td>
<td>CYP1A2, CYP2C19, CYP2D6</td>
<td>CYP2D6 [4,5,13]</td>
</tr>
</tbody>
</table>
risperidone  CYP2D6, CYP3A4 [2,5]
flunitrazepam  CYP2C19, CYP3A4 [17]
acetaminophen  CYP1A2, CYP2E1 [5]

* The number in brackets indicate the number of references.

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, 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.

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.

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. 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 have been clinically reported. Based on previous evidence, it is speculated that the drug levels in the present case might have been elevated due to inhibition of CYP2D6 by drugs such as levomepromazine, promethazine, and clomipramine, and of CYP3A4 and CYP1A2 by levomepromazine. 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. Estazolam is a
triazolobenzodiazepine derivative that is metabolized by CYP3A4, and is prescribed for the short-term management of insomnia; somnolence, respiratory depression, and coma are observed with its overdose. In the current case, blood estazolam level might also have been elevated due to the inhibition of CYP3A4.

We speculate that accumulation of dextromethorphan and estazolam in the present case’s blood was possibly due to an inhibitory pharmacokinetic drug interaction. Thus, these two drugs might also have contributed to her death. The present case shows that more attention should to be paid to interactions between multiple psychotropic drugs.

**Conclusion.** We concluded that the cause of her death was 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.

**References**


