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CASE REPORT

Discrimination Between Drug Abuse and Medical Therapy

Case report of a tranylcypromine overdose-related fatality

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التمييز بين تعاطي المخدرات والعلاج الدوائي

تقرير حالة عن وفاة ذات صلة باستعمال جرعة زائدة لعقار الترانيلسيبرومين

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ABSTRACT: Tranylcypromine is an effective antidepressant from the class of monoamine oxidase inhibitors and is structurally related to amphetamine. However, reports differ regarding the potential metabolism of tranylcypromine to amphetamine and methamphetamine within the human body. We report a 25-year-old woman with severe depression who died due to a fatal tranylcypromine overdose in 2016. She had been prescribed tranylcypromine one day previously and had no history of previous suicide attempts or substance abuse. The body was transferred to a forensic medicine department in Tehran, Iran for the autopsy. A urine sample was positive for tranylcypromine, amphetamine and methamphetamine using gas chromatography/mass spectrometry after derivatisation with heptafluorobutyric acid. As amphetamines were present in the urine sample, it was assumed that the tranylcypromine had been converted to amphetamines metabolically. As such, it is possible that the legitimate use of certain prescription drugs may complicate the interpretation of test results for illegal drugs.

Keywords: Tranylcypromine; Amphetamine; Metabolism; Forensic Toxicology; Substance Abuse Detection; Case Report; Iran.

الملخص: عقار الترانيلسيبرومين المضاد للاكتئاب هو من فئة مثبطات مونو أمين أوكسيديز و يشابه هيكليا الأمفتامين. توجد تقارير مختلفة لعملية أيض الترانيل سيبرومين اثناء تحويله إلى أمفيتامين و ميثا مفيتامين. هذا تقرير عن إمرأة يبلغ عمرها 25 عاما كانت تعاني من الاكتئاب الشديد توفت بسبب تناول جرعة زائدة مميتة من عقار الترانيل سيبرومين عام 2016م. تم وصف عقار الترانيل سيبرومين لها قبل وفاتها بيوم واحد. أظهر التاريخ المرضي لها عدم وجود سابقة انتحار أو ادمان المخدرات. تم نقل الجثمان إلى قسم الطب الشرعي في طهران-إيران بغرض التشريح. بعد اشتقاق عينة البول تم الكشف عن وجود الترانيل سيبرومين، ميثا مفيتامين و الأميفتامين باستخدام طريقة الكروماتوجرافي الغازي/التحليل الطيفي. وقد افترض أن الأمفيتامين ناتج عن أيض عقار الترانيل سيبرومين. بناء على هذا فأن استعمال بعض الأدوية المرخصة قد يسبب مشاكل في تأويل نتائج فحص العقاقير الممنوعة.

الكلمات المفتاحية: ترانيل سيبرومين؛ أمفيتامين؛ أيض؛ علم السموم الشرعي؛ التعرف على ادمان المواد؛ تقرير حالة؛ إيران.

Tranylcypromine is an irreversible non-selective monoamine oxidase inhibitor often prescribed as an antidepressant.[1,2] Tranylcypromine is a well-known structural analogue of amphetamine to which a cyclopropyl ring is fused; however, there is some evidence that suggests the cyclopropyl ring undergoes metabolic cleavage to form three metabolites: amphetamine, 1-amino-2-phenylpropane and 1-amino-3-phenylpropane.[1,3] This metabolic formation of amphetamine is responsible for the therapeutic effects of tranylcypromine as well as those that occur following withdrawal.[1–4] A therapeutic dose of tranylcypromine constitutes 30 mg per day, usually given in divided doses. While this amount of the drug is unlikely to produce a marked degree of amphetamine-like central activity, the contribution of amphetamine to the pharmacological response should not be underestimated.[5]

Reliable laboratory methods of detecting drugs such as tranylcypromine and amphetamine-type stimulants have been previously validated using various parameters, such as selectivity, linearity, accuracy and precision, limits of detection, limits of quantitation and recovery.[6,7] Most countries legally restrict the recreational use of amphetamines and categorise this class of drugs under controlled substances.[8] Despite this, the use of amphetamine-type stimulants has become increasingly popular, particularly among young people;[7] it is therefore important to accurately interpret any forensic toxicology results in both living and deceased patients. In addition, physicians should be aware of the possibility

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that certain therapeutic prescription drugs—such as tranylcypromine—may be metabolised to illegal drugs like amphetamines in humans. This report describes a case of a fatal overdose involving tranylcypromine, wherein tranylcypromine, methamphetamine and amphetamine were detected postmortem.

Case Report

A 25-year-old woman was found dead in her bed in Tehran, Iran, in 2016. She had last been seen alive five hours previously. She had a history of severe depression, but there was no record of any prior suicide attempts. In addition, her relatives reported that she had no history of substance abuse. One day before her death, she had been prescribed tranylcypromine by her psychiatrist, to be taken twice a day. Upon investigation of the scene of death, the police found a bottle of 100 tablets containing 10 mg of tranylcypromine each beside her bed, of which 19 tablets appeared to be missing. The corpse was subsequently transferred to the Department of Forensic Medicine at the Legal Medicine Organization in Tehran for an autopsy in order to determine the cause of death.

The autopsy revealed no external injuries to the body. The woman weighed 60 kg and her height was 163 cm. The internal organs were normal in size and shape. The stomach contained approximately 300 mL of fluid and some digested food. Under the supervision of a forensic specialist, biological specimens were collected, including liver, stomach content, vitreous humour, blood and bile samples. Solid tissue, such as that of the liver, was minced and homogenised with two parts of distilled water. Liquid-liquid extraction with chloroform and isopropanol (at 80:20 volume/volume percent [v/v]) was used to prepare the biological matrices before analysis. Extraction products were subsequently analysed via thin-layer chromatography, high-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GCMS).

Thin-layer chromatography was performed with 20 x 20 cm pre-coated aluminium sheets of 0.25 mm silica gel layer thickness with an ultraviolet fluorescent indicator (ALUGRAM® Xtra SIL G SIL UV254, Macherey-Nagel Gmbh, Düren, Germany) whereas HPLC was performed with a diode array detector using a Eurospher II 100-5 C18 column of 250 mm x 4.6 mm at 5 µm particle size and 100 Å pore size (Knauer, Berlin, Germany) with a pump (Smartline 1000, Knauer). GCMS was performed using the 7890A Gas Chromatograph and 5975C Mass Selective Detector (Agilent Technologies Inc., Santa Clara, California, USA). Blood and vitreous humour samples were analysed quantitatively to detect ethanol and methanol via headspace gas chromatography using the 6890N Network Gas Chromatograph (Agilent Technologies Inc.) equipped with a flame ionisation detector. A spectrophotometer (Series 9000, Cecil Instruments, Peterborough, UK) was used to analyse carboxyhaemoglobin levels.

Amphetamine and methamphetamine levels in the urine sample were analysed using the derivatisation reagent heptafluorobutyric acid (HFBA).[6,7] A total of 1 mL of urine was centrifuged for five minutes at 9,000 rpm and the supernatant was separated. The sample was alkalinised by adding 0.5 M of potassium hydroxide and the pH was adjusted to 11–12. Amphetamine and methamphetamine were extracted by adding 3 mL of n-hexane and shaking the sample for 20 minutes before immersing it in a freezing bath to separate the organic compounds. A freezing bath was used instead of more common methods, such as a separatory funnel, because of the low volume of urine in the sample, which made it more difficult to distinguish between aqueous and organic phases. In order to avoid losing volatile analytes such as amphetamine and methamphetamine, a small amount of hydrochloric acid (HCl) was added before the evaporation stage as a keeper solvent.[9] A total of 100 µL of methanol and HCl (at a ratio of 99:1 v/v) was added to the medium and the solvent was completely evaporated under a gentle stream of nitrogen. The extract was reconstituted with 100 µL of n-heptane and vortexed for one minute. For derivatisation, 25 µL of HFBA was added and the mixture was vortexed for three minutes. The excess HFBA was deactivated by adding 500 µL of sodium bicarbonate and vortexing the mixture for one minute. The mixture was again placed in a freezing bath and the organic layer was separated for GCMS analysis.

Instrument control and data acquisition was performed using ChemStation software, Version G20-70BA (Agilent Technologies Inc.) with a 5-ms capillary column at a length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 mm. Gas chromatographic conditions were set as follows. Helium of 99.99% purity was used as a carrier gas with a constant flow rate of 1.5 mL per minute. The inlet temperature was set at 250 °C and a volume of 1 mL was used for the splitless injection. The column temperature was set at 90 °C for one minute and then increased at a rate of 20 °C per minute to 280 °C for five minutes. Mass source and quadrupole temperatures were fixed at 230 °C and 150 °C, respectively. The ion source was operated by electron impact at 70 eV in selected ion monitoring and full-scan mode with a scan range of

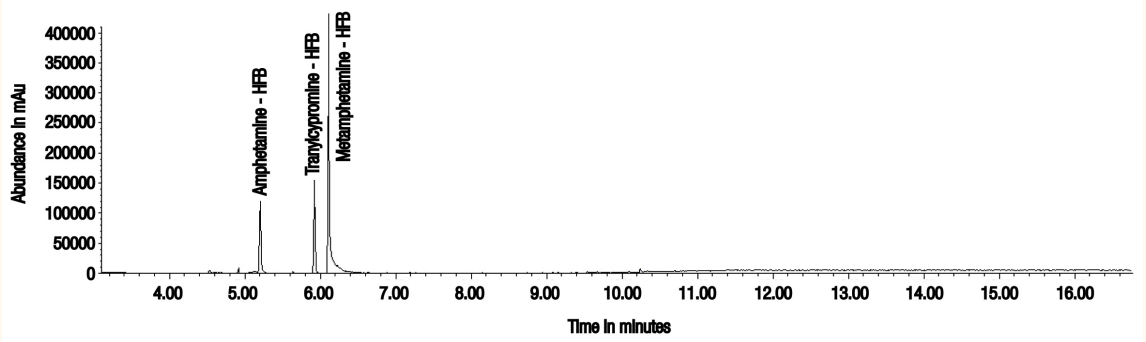

Figure 1: Chromatogram of amphetamine, methamphetamine and tranylcypromine derivatised with heptafluorobutyric acid as detected in the urine of a 25-year-old woman who died due to a fatal tranylcypromine overdose.
HFB = heptafluorobutyric acid.

40–500 m/z. The toxicological results of the urine sample were negative for all drugs except for tranylcypromine, amphetamine and methamphetamine [Figures 1 and 2]. Tranylcypromine was also detected in the stomach contents.

Discussion

Among humans, severe intoxication can occur if the maximum daily dose of 60 mg of tranylcypromine is exceeded.[1] Tranylcypromine-related deaths can be attributed to either an overdose due to misuse of the drug by the patient or to a hypertensive crisis leading to a cerebrovascular accident as a result of a therapeutic dose.[10] Although tranylcypromine has been commercially available for many years, little is yet known of its metabolic pathway. Baker *et al.* reported finding N- acetylcysteine and ring-hydroxylated metabolites in the brain of a rat following the administration of tranylcypromine.[11] However, there are conflicting reports as to whether tranylcypromine is metabolised to amphetamine and methamphetamine.[3,4,8,12,13]

In the current case, tranylcypromine was detected in combination with amphetamine and methamphetamine in the urine sample of a fatal overdose case. One possible explanation for the presence of amphetamines in such postmortem specimens is the biotransformation of tranylcypromine to amphetamine and methamphetamine within the human body. Crifasi *et al.* similarly reported the presence of metabolic products, such as amphetamine and methamphetamine, in the body of a tranylcypromine overdose case.[14] Youdim *et al.* also detected amphetamine in the plasma of a patient who had overdosed on tranylcypromine.[4] However, Sherry *et al.* found no amphetamines in biological samples obtained from both rats and

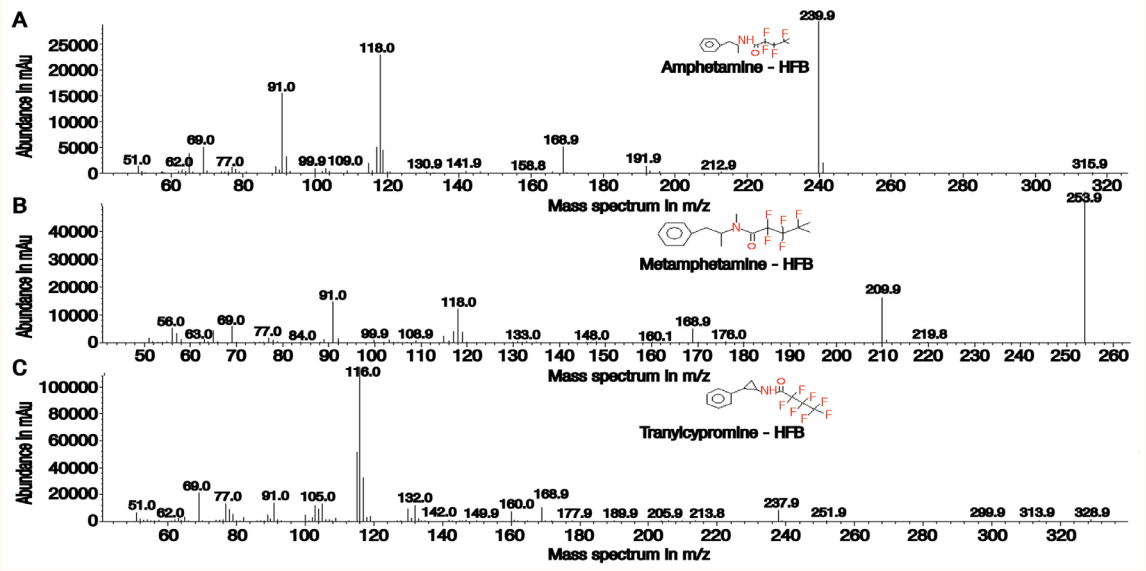

Figure 2: Mass spectra graphs of (**A**) amphetamine, (**B**) methamphetamine and (**C**) tranylcypromine derivatised with heptafluorobutyric acid as detected in the urine of a 25-year-old woman who died due to a fatal tranylcypromine overdose.

humans following tranylcypromine administration.[3] Other studies have also not detected amphetamine or methamphetamine in the biological samples of tranylcypromine overdose cases.[15,16]

According to Yonemitsu *et al.*, amphetamine and methamphetamine can be identified as metabolic products following a tranylcypromine overdose as these metabolites produce symptoms of poisoning; however, with therapeutic doses of tranylcypromine, amphetamine and methamphetamine cannot be detected.[10] In another study, 5 mg/kg of tranylcypromine-14C was injected into animals in which approximately 4% of the injected drug was excreted unchanged and 12% was excreted as hippuric acid; in contrast, when amphetamine was injected, these values changed to 15% and 2%, respectively.[12] As such, the authors concluded that the opening of the cyclopropyl ring in tranylcypromine did not involve the formation of amphetamine.[12] Ragab *et al.* noted that tranylcypromine is relatively stable on exposure to ultraviolet light and that only small amounts of degradation products can be detected using reversed-phase HPLC; the authors concluded that the cyclopropyl ring opens in both acidic and alkaline conditions and that the degradation products have different chemical structures from amphetamine and methamphetamine.[17]

One possible explanation for the discrepancies in the findings of the aforementioned studies may be individual variations in drug metabolism among patients. For example, ethnic variations in cytochrome P450 isoenzymes have resulted in differing levels of metabolic activity, clinical responses and drug interactions.[18,19] Furthermore, variations in drug metabolism between individuals may also be the reason for contradictory results when biological samples are analysed to detect a parent drug and its metabolites.[20] Additionally, the pharmacokinetic characteristics of certain drugs may be altered in an overdose situation.[19,21] Therefore, a number of confounding variables can influence the interpretation of postmortem forensic toxicology results. As such, details of a specific case can be crucial to forensic toxicologists, particularly when interpreting analytical toxicology results and seeking to determine the source of the drugs found. As there are conflicting data regarding the production of amphetamine and methamphetamine in tranylcypromine overdose cases, further studies among larger samples are needed to confirm the *in vivo* biotransformation of tranylcypromine to amphetamine and methamphetamine. Unfortunately, in the current case, due to the limited number of tranylcypromine overdose-related fatalities in Iran, it was not possible to publish a case series on this topic.

Conclusion

The present report described a fatal tranylcypromine overdose case in which methamphetamine and amphetamine were found in the urine samples of a young woman with no history of substance abuse. In this case, it is conceivable that the tranylcypromine underwent a metabolic opening of the cyclopropyl ring to produce amphetamine and methamphetamine. However, further investigation is needed to determine the exact metabolic pathway of tranylcypromine in the human body. This would provide valuable evidence when interpreting forensic toxicology results so as to ascertain whether the presence of methamphetamine and amphetamine indicate the legitimate use of a prescription drug or, alternatively, illegal substance abuse.

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