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Toxicological Findings in a Possible Drug-drug Interaction Death: A Case Report

النتائج السمية في الوفيات المحتملة الناتجة عن التداخلات الدوائية: تقرير حالة

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Abstract

Synergistic effects are the most encountered types of drug-drug interaction in post-mortem toxicology. Concomitant use of fentanyl, tramadol and carbamazepine may increase the risk of severe serotonin toxicity. The decedent was a 32-year-old black man, with a history of severe migraine headaches. He died after being administered several drugs to treat the migraine. For fentanyl identification and quantification, samples were extracted using solid phase extraction and analyzed by GC-MS. For carbamazepine and tramadol identification and quantification, samples were extracted by liquid-liquid extraction and analyzed by LC-QTOF. Toxicology showed post-mortem concentrations of fentanyl 0.033, 0.025, 0.005, 0.0127, and 0.005 mg/L; tramadol 0.143, 0.093, 0.043, 0.09, and 0.08 mg/L; carbamazepine 1.6, 1.04, 0.3, 0.83, and 0.18 mg/L in the blood, brain, liver, kidney and stomach, respectively. In this case report, the combination of serotonergic drugs can contribute to synergistic serotonergic effects. Therefore, drug-drug interaction is expected, and the cause of death may be attributed to toxic synergistic drug-drug interaction including fentanyl, tramadol and carbamazepine.

Keywords: Forensic Science, Post-mortem Toxicology, Drug-drug Interaction, Fentanyl, Tramadol, Carbamazepine.

المستخلص

تعد التأثيرات التآزرية أكثر أنواع التداخلات الدوائية الشائعة في علم السموم بعد الوفاة. وربما يزيد استخدام الفينتانيل، والترامادول، والكاربامازيبين معًا من خطورة سمية السيروتونين. كان المتوفى شخصًا عمره 32 عامًا ذا بشرة سمراء، وله تاريخ مرضي بالصداع النصفي الحاد. وقد تُوفي هذا الشخص بعد تناوله عدة عقاقير لعلاج الصداع النصفي.

وللكشف عن الفينتانيل وقياس كميته، تم تحضير العينات عبر الاستخلاص باستعمال الطور الصلب وتحليلها من خلال جهاز كروماتوغرافيا الغاز المقترن بمطياف الكتلة (GC-MS). وللكشف عن الكاربامازيبين والترامادول وقياس كميتهما، تم تحضير العينات عبر الاستخلاص سائل - سائل وتحليلها بواسطة جهاز الكروماتوغرافيا السائلة المقترنة بمطياف الكتلة بتقنية زمن الارتحال (LC-QTOF). وأظهرت التحاليل السُمّية تركيزات ما بعد الوفاة للفينتانيل بمقدار 0.033، و0.25، و0.005، و0.0127، و0.005، ملجم/لتر؛ وللترامادول 0.143، و0.093، و0.043، و0.09، و0.08 ملجم/لتر، وللكاربامازيبين 1.6، و1.04، و0.3، و0.83، و0.18 ملجم/لتر في كل من الدم، والمخ، والكبد، والكليتين، والمعدة، على التوالي. ويوضح تقرير الحالة هذا أن مجموعة مؤلفة من أدوية السيروتونين يمكن أن تساهم في حدوث آثار تآزرية للسيروتونين. لذلك، يعد تداخل دواء مع دواء آخر أمرًا متوقعًا، وربما يرجع سبب الوفاة إلى تداخل سُمّي تآزري بين أدوية منها فينتانيل، ترامادول، وكاربامازيبين.

الكلمات المفتاحية: علوم الأدلة الجنائية، علم السموم ما بعد الوفاة، التداخل الدوائي، فينتانيل، ترامادول، كاربامازيبين.

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1. Introduction

Multi-drug use is a proven to be a risk factor for drug-drug interaction and may lead to severe adverse reactions and fatality [1, 2]. It is common in drug poisoning deaths [2]. Several issues like drug-drug interaction, redistribution, sampling time, enzymatic activity, and drug stability, make the interpretation of post-mortem toxicological data challenging [3]. The occurrence of drug-drug interaction is highly common in hospitalized elderly patients and may lead to death [4]. Fentanyl, tramadol, and carbamazepine are common in multi-drug poisoning deaths [2, 5]. This study focuses on a death in which fentanyl, tramadol and carbamazepine were detected.

2. Case report

The decedent was a 32-year-old black male, with a history of severe migraine headaches. He died after being administered several drugs to treat the migraine. The decedent was discovered dead in his room. Post-mortem forensic toxicology was performed on the blood, brain, liver, kidneys and stomach. For the determination of fentanyl, samples were extracted using solid phase extraction and analyzed by GCMS using the previously described method [6]. Carbamazepine and tramadol were identified and quantified using a quadrupole time-of-flight mass spectrometry (Q-TOF-MS) (X500R Q-TOF from Sciex, USA) coupled with the Sciex UPLC system using the SCIEX vMethod [7]. Briefly, 100 μL of homogenized tissue samples were mixed with 900 μL of methanol water mixture (7:2) in an Eppendorf tube, vortexed for 0.5 min and centrifuged for 5 mins at 10000 rpm. The supernatant was transferred into an LC vial for analysis. For the blood, 100 μL of the samples were mixed with 900 μL of methanol acetonitrile mixture (5:5) in an Eppendorf tube, vortexed for 1 min and sonicated for 3 mins. After sonication, samples were vortexed for 1 min and centrifuged for 5 mins at 10000 rpm. The supernatant was transferred into a clean Eppendorf tube and dried under nitrogen gas. The dried material was reconstituted with 500 μL of methanol water mixture (7:2), vortexed for 0.5 min, and centrifuged for 5 mins at 10000 rpm. The supernatant was transferred into LC vial for analysis. Phenomenex Kinetex 2.6 Phenyl-Hexyl 100 Å column (50 × 4.6 mm I.D) (Phenomenex) was carried out at ambient temperature. The mobile phase consisted of 0.05% formic acid in methanol (A) and 10 mM ammonium formate buffer (B) and was delivered in gradient mode according to the Sciex vMethod.

The analytes were analyzed at Q-TOF using positive ESI and full scan mode. The analytes were identified by the system using four identification criteria including; mass error, retention time, isotope and library confidence, which were confirmed according to the standard of each analyte. For quantitative analysis, a calibration curve was established for each analyte using serial dilutions of each analyte standard. Fentanyl was positive at 0.033, 0.025, 0.005, 0.013, and 0.005 mg/L in the blood, brain, liver, kidneys, and stomach respectively, by GC-MS. Tramadol was positive at 0.143, 0.093, 0.043, 0.09, and 0.08 mg/l in the blood, brain, liver, kidneys and stomach respectively, by liquid chromatography, coupled with LC-QTOF-MS. Carbamazepine tested positive at 1.6, 1.04, 0.3, 0.83, and 0.18 mg/L in the blood, brain, liver, kidneys and stomach respectively, by LC-QTOF. In this case report, a combination of serotonergic drugs could have contributed to synergistic serotonergic effect. Therefore, drug-drug interaction was expected, and the cause of death may be attributed to mixed drug toxicity including fentanyl, tramadol and carbamazepine.

3. Discussion

This case is an example of possible drug-drug interaction including fentanyl, tramadol, and carbamazepine. Fentanyl is a synthetic opioid agonist used to control pain. Fentanyl acts on the mu opioid receptor. These mu-binding sites are found in the spinal cord, brain, and other tissues [8]. Tramadol is a synthetic opioid analgesic. It is used to relieve moderate to severe pain. Tramadol and its active metabolite O-desmethyltramadol acts on the mu opioid receptor in a similar way to fentanyl, and through the weak inhibition of norepinephrine and serotonin reuptake [9]. Carbamazepine is used as an anticonvulsant drug, as well as in the treatment of trigeminal neuralgia, by a mechanism of action that is unknown [10].

As can be seen from the above mechanism of action, fentanyl and tramadol act on the same binding site and exert similar effects and have a synergistic effect. This will increase the risk of respiratory and CNS depressant effect which may lead to hypotension, sedation, coma and even death [11]. In a clinical setting, this drug combination should be avoided unless other alternatives are inadequate. If this combination is used, then small doses, short duration and close monitoring for respiratory depression, CNS depressants, and serotonin syndrome are required.

In addition, drugs that affect CYP3A4 function should be used with caution when given along-with fentanyl and/or tramadol to avoid drug-drug interactions. In this regard, carbamazepine is an enzyme inducer of CYP3A4 so its concomitant use with fentanyl and tramadol will decrease their effects [12, 13]. This interaction will lead to lack of efficacy, or possibly to the development of a withdrawal syndrome in a patient who has developed physical dependence on fentanyl. After stopping carbamazepine, the induction effect decreases and consequently the fentanyl plasma concentration increases. This may prolong or increase both the therapeutic and adverse effects of fentanyl.

Furthermore, carbamazepine as an enzyme inducer will decrease the level of tramadol and its active metabolite O-desmethyltramadol [12, 14]. Therefore, the concomitant use of tramadol with the serotonergic CYP3A4 inducer will lead to an increase in the risk of serotonin syndrome and reduce the level of tramadol. Also, tramadol use has been associated with seizures. Therefore, if the patient stops taking carbamazepine, the concentration of tramadol and its active metabolite may increase, consequently increasing the risk of seizures and respiratory depression or sedation.

Concurrent use of carbamazepine, tramadol, and fentanyl may be life-threatening and increase the risk of respiratory depression and serotonin syndrome. Table-1 shows the quantification of each drug in the decedent's samples, along with the established therapeutic, toxic, and lethal ranges [15].

In the present case, the post-mortem drug levels are within the nontoxic range, with the exception of fentanyl, which reached the lethal range according to established lethal levels (Table-1). Generally, the best post-mortem samples for confirmation and quantification are vitreous humor, femoral blood, and subclavian blood since these materials are less prone to post-mortem redistribution. Brain tissue is also less prone to post-mortem redistribution and it was found that tramadol levels in the brain were nearly equal to vitreous humor [16]. Although the brain fentanyl level was within the lethal range, the interpretation of the result is challenging to clarify whether it is considered contributory or noncontributory to the present case death [17].

Different factors should be considered in this case (1) drug-drug interaction: kinetic and dynamic interaction. For example, the CYP3A4 enzymatic

Table 1- *Concentrations of each drug in post-mortem samples.*

Drug	Matrix	Measured Levels	Therapeutic Range*	Toxic Range*	Lethal Range*
Tramadol (mg/L)	Blood	0.143	0.1 - 3.0	1-24	1.3-89
	Brain	0.093	--	--	44
	Liver	0.043	0.3	--	6.2-69
	Kidney	0.09	0.4	--	3-37
	Stomach	0.08	--	--	--
Fentanyl (mg/L)	Blood	0.033	0.0002-0.07	0.003-0.02	0.003-0.2
	Brain	0.025	0.003-0.01	--	0.01-0.1
	Liver	0.005	0.008-0.2	--	0.004-0.4
	Kidney	0.0127	--	--	0.01-0.09
	Stomach	0.005	--	--	--
Carbamazepine (mg/L)	Blood	1.6	1.9 - 19	10-55	20-73
	Brain	1.04	--	--	78-86
	Liver	0.3	2.2	--	123
	Kidney	0.83	--	--	72
	Stomach	0.18	--	--	--

** Therapeutic, toxic and lethal ranges are cited from the reference [13].*

genotype that is involved in drug metabolism and may help to confirm drug poisoning. CYP3A4 is involved in metabolic pathways of carbamazepine, fentanyl, and tramadol [18]; (2) degree of tolerance, which is dependent on the decedent`s medical history. Tolerance can develop with carbamazepine, fentanyl, and tramadol; therefore, it should be considered when interpreting post-mortem drug levels [19]. In this regard, parent drug to its metabolite ratio in blood may provide some insight into tolerance status at the time of death and may help to clarify the contribution of drugs to the decedent's death [19]. While parent drug to its metabolite urine ratio may provide valuable information about the recent intake of drugs; (3) the effect of post-mortem redistribution that lead to a variation in drug levels according to the interval between death and specimen collection. Basic and lipophilic drugs, with a high volume of distribution, are more prone to post-mortem redistribution [20].

Fentanyl is a basic and lipophilic drug (pKa: 8.4), with a moderate to high volume of distribution (Vd: 3-8 l/kg), and thus, it is prone to post-mortem redistribution [8, 21]. Tramadol is a basic lipophilic drug (pKa: 9.4), with a moderate volume of distribution (Vd: 2.6-2.9 l/kg), and thus, it is moderately prone to post-mortem redistribution [9, 21]. Thus, the post-mortem levels of fentanyl and tramadol do not necessarily reflect the levels at the time of death. While carbamazepine is an acidic drug (pKa: 2.3) with a relatively small to moderate volume of distribution (Vd: 0.8-1.8 l/kg), it is apparently not sub-

ject to post-mortem redistribution [10, 21]. Thus, the post-mortem carbamazepine levels may reflect the levels at time of death.

In this case report, the combination of carbamazepine, fentanyl, and tramadol may have contributed to serotonin poisoning (dynamic drug-drug interaction) that may lead to fatal poisoning. Several studies demonstrated that carbamazepine, fentanyl, and tramadol contribute to cause serotonin toxicity, either used alone or in concomitant use with other serotonergic agents [22]. These drugs are more likely to produce dynamic drug-drug interaction and may cause severe serotonin toxicity, as well as death [23]. In addition, it could be speculated that the combination of these drugs may have induced respiratory depression and cardiac arrest that led to death [24]. Further studies are needed to examine the effect of dynamic drug-drug interactions in forensic settings, to overcome the scarcity of post-mortem studies of drug-drug interaction and clarify the potential role they play in causing death.

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Conflict of interest

None

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