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Homicidal Poisoning of Heroin and Estazolam: Autopsy and Pathological Findings, Toxicological Analysis

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Abstract

We reported an unusual homicidal case in which a 40-year-old woman was deceived into drinking a cup of milk that had 72 tablets of estazolam (2mg/tablet) dissolved in, and then being injected heroin aqueous solution on the right deltoid region by the criminal. At autopsy, pinpoint pupils and a new injection site on the right deltoid region were found. The pathologic pictures showed multiple patchy hemorrhages and considerable amounts of foreign amorphous substance with yellow appearance at the injection site. Some double refracting crystals with the forms of Maltese cross, acicular, rhomb or irregular were found by polarizing microscope, which may result from the diluent in heroin such as starch. Toxicological qualitative analysis by gas chromatography mass spectrometry (GC-MS) demonstrated the presence of benzodiazepine and morphine in blood and urine, and heroin in the injector left at scene. Quantitative analysis was also performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), providing the data on distribution of 6-monoacetylmorphine, morphine and estazolam in the woman's body. And the cause of death was determined to polydrug heroin-related deaths due to the combined poisoning of heroin and estazolam. It taught a lesson that the determination of other drugs, particularly central nervous system depressants in heroin poisoning were quite important in forensic expertise.

Keywords: *Heroin, Estazolam, Homicide, LC-MS/MS*

1. Introduction

Heroin (3, 6-diacetylmorphine) that was first synthesized from morphine in 1874 has been as a leading worldwide cause of morbidity and mortality due to substance abuse. In certain countries, heroin is used legally for treating chronic pain and other medical conditions [15]. However, because of its severe toxicity and the risk of addiction and dependence, heroin has been responsible for much accidental intoxication as well as homicidal poisonings since its discovery [1, 13]. The chemical addition of ester groups to heroin yields lipophilicity. Therefore, heroin may pass the blood-brain-barrier much faster than its precursor morphine [16], contributing to a more intense pharmacodynamic effect with more immediate onset

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of action compared to morphine. It undergoes rapid hydrolyzation in human blood by sequential deacylation of two ester bonds to yield first 6-monoacetylmorphine (6-MAM), which is further deacetylated to morphine at a somewhat slower rate [20]. The serum enzyme butyrylcholinesterase performs the first step, with a catalytic efficiency of 4.5/min per μmol/L [24]. The second step mainly occurs in the liver [4] where glucuronides are conjugated to the 3 and 6 positions of morphine [11]. Heroin acts by interaction with G protein-coupled opioid receptors on presynaptic nerve terminals in the brain, including the μ, κ and Δ receptors. It opens K+ channels inhibiting postsynaptic neurons, resulting in euphoria, pinpoint pupils and respiratory depression [18].

Estazolam (Prosom) is a triazolobenzodiazepine derivative that is similar structurally to alprazolam and triazolam. It is an intermediate-acting benzodiazepine hypnotic, indicated for the short-term management of insomnia [5]. Binding to the GABAA receptor, the benzodiazepine locks the GABAA receptor into a high affinity conformation, increasing the opening frequency of chloride ion channel contributing to membrane hyperpolarization. As a result, the inhibitory effect of the available GABA increases, leading to sedation, respiratory depression [18].

Here, we reported an interesting case in which homicide was revealed by investigation of the case, autopsy, pathological examinations and toxicological qualitative analysis by gas chromatography mass spectrometry (GC-MS). Quantitative analysis was also performed by liquid chromatography-tandem mass spectrometry (LC-MS/MS), providing some important data on the distribution of 6-MAM, morphine and estazolam in human body.

2. Materials and Methods

2.1. Case Report

A 40-year-old woman was found dead in bed at 9:00 p.m, with a 2.5 mL plastic syringe laid aside her. Interestingly, a patchy hemorrhage on the right deltoid region was observed, with a new injection site at the corresponding location under the clothes (Figure1). The case investigation showed that approximately at 6:00 p.m., the decedent was deceived into drinking a cup of milk that had 72 tablets of estazolam (2 mg/tablet) dissolved in. About 10 minutes later, she was injected an injection of aqueous solution that had dissolved 1 g heroin on the right deltoid region by the criminal. And then the decedent showed the absence of breath and heartbeat at about 6:55 p.m. Homicide was confirmed by the systematic professional investigation of the case, revealing the absence of substance abuse and motive of suicide for the decedent.

2.2. Autopsy and Pathological Examinations

Routine forensic autopsy was performed about 36 h after death by the Faculty of Forensic Medicine, HUST (China). The autopsy material was fixed in 10% formalin for subsequent staining of haematoxylin-eosin (HE). Skin of the injection site was detected by polarizing microscope examinations further.

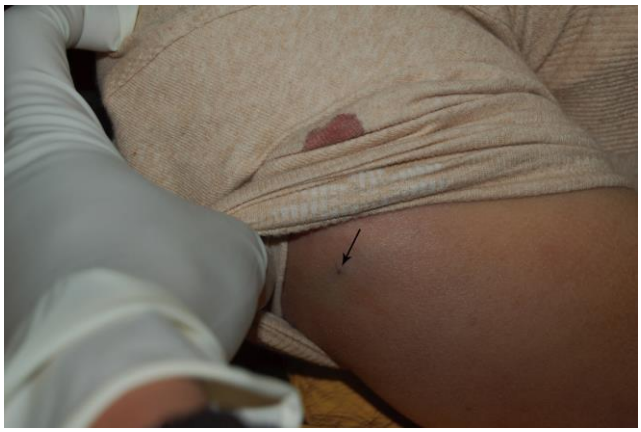

Figure 1. The Injection Site on Right Deltoid Region. There Was a Patchy Hemorrhage on the Right Deltoid Region, a New Injection Site (Arrow) under the Clothes and Cyanosis on the Skin

2.3. Toxicological Analysis

Toxicological qualitative and quantitative analysis were performed by GC/MS (5973N, Angilent, USA) and LC-MS/MS (API 4000, Applied Biosystems, USA), respectively. Stock standard solutions at a concentration of 1.0 mg/mL were prepared in methanol, using standard samples of heroin, 6-MAM, morphine or estazolam. Working solutions at the concentration of 1.0 μL/mL were prepared by dilution of the stock standards and stored at -20℃ until analysis. The deuterated internal standard (I.S.) working solution was used at a concentration of 10 μg/mL. Whole blood from heart, urine, skin and muscle tissue from the injection site, brain, heart, lung, liver and kidney were extracted according to the following procedure: 1.0 mL of each liquid sample or 1.0 g of each tissue homogenate with 50 μl of I.S. working solution was transferred into 25 ml screw-capped glass tubes and 3 ml of methanol was added. The tubes were placed in a horizontal shaker for 20 min. After centrifugation at 2000 rpm for 10 min the organic layer was dried under nitrogen. The dried extracts were then dissolved in 1 ml borax buffer, pH 9.2 and applied for detection. Aliquots of 1.0 μL and 5.0 μL were injected into the system of GC/MS and LC-MS/MS, respectively.

3. Results and Discussion

3.1. Autopsy and Pathological Examinations Findings

Bilateral miosis with diameters about 1 mm was found and cyanosis in the fingernails and toenails was also conspicuous in autopsy. The pathological examinations showed multiple patchy hemorrhages and considerable amounts of foreign amorphous substance with yellow appearance at the injection site skin (Figure 2), where some double refracting crystals with the forms of Maltese cross, acicular, rhomb, or irregular were also found by polarizing microscope examinations (Figure 3). The brain was marked edema and congestion, weighing 1360 g. Considerable production of white mucus was visible in the main bronchi. The lungs were a bit heavier than normal, the left lung weighing 510 g and right lung 616 g. Obvious lung edema and congestion, as well as focal hemorrhage were observed in the lungs. Importantly, gastric content was still discernible, weighing about 400 g. No gross abnormalities of the external and cut surfaces, as well as fatal pathological changes were observed in the heart, liver, kidney and other organs.

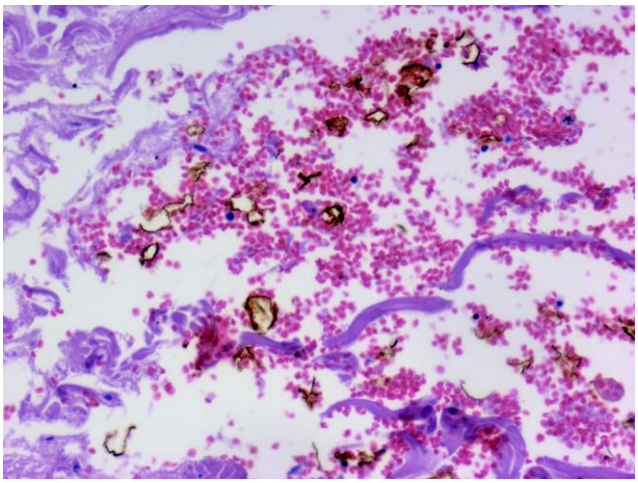

Figure 2. Hemorrhage and Amorphous Yellow Substance at the Injection Site Skin. There were Multiple Patchy Hemorrhages and Considerable Amounts of Foreign Amorphous Substance with Yellow Appearance at the Injection Site (Hematoxylin and Eosin Staining; Original Magnification ×200)

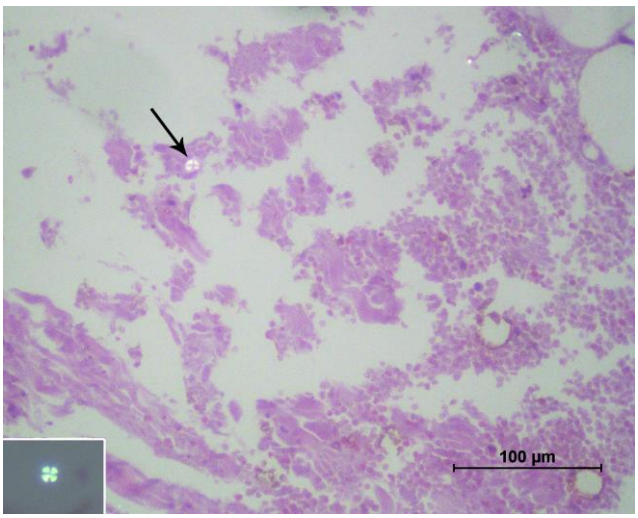

Figure 3. Double Refracting Crystals with the Forms of Maltese Cross at the Injection Site (Hematoxylin and Eosin Staining; Polarizing Microscope)

3.2. Toxicological Analysis Results

GC/MS analysis demonstrated the presence of benzodiazepine and morphine in blood and urine, but absence of alcohol, barbiturates, organophosphorus pesticides and tetramine. Moreover, heroin was also determined in the injector.

The LC-MS/MS conditions (Table 1) include: M+1, Daughter ion, DP (V), CE (eV), retention time (tR) and limit of detection (LOD). And the results were shown in Table 2. The skin tissue had the maximum concentration of 6-MAM (353 ng/g), while that of the muscle from the injection site showed a little bit lower. Mild 6-MAM aggregation was discovered in the brain, with an average concentration of 178 ng/mL, which was lower than that of liver, lung and kidney. Remarkably, the blood concentration of 6-MAM was lower than all the samples but myocardium. Also, the skin tissue showed the maximum concentration of morphine (31206 ng/g), while the minimum appeared in the brain (average 223 ng/g). The concentrations of morphine in the liver (1179 ng/g) and kidney (1781 ng/g) were much higher than that of blood (643 ng/mL). The aggregation of estazolam was found in the liver (1080 ng/g)

comparing with 797 ng/mL in the blood, followed by the lung, kidney, muscle, myocardium and brain.

Table 1. Settings on LC-MS/MS for Detecting 6-MAM, Morphine and Estazolam

Agent	M+1	Daughter ion	DP(V)	CE(eV)
6-MAM	328.1	211.3	90	36
Morphine	258.2	201.2	80	36
Estazolam	295.2	267.3	70	34

Table 2. Distribution of 6-MAM, Morphine and Estazolam in Different Tissues (ng/g)

Specimens	6-MAM	Morphine	Estazolam
Skin tissue[a]	353	31206	1132
Muscle[a]	312	301	352
Blood (ng/mL)	101	643	797
Frontal lobe	222	252	389
Occipital lobe	254	233	349
Cingulate gyrus	170	166	269
Corpus callosum	116	108	247
Epencephalon	157	429	384
Brainstem	147	149	316
Liver	325	1179	1080
Kidney	293	1781	433
Lung	306	934	600
Myocardium	81	1118	353

The skin and muscle specimens were from the injection site on the right deltoid region.

3.3. Cause of Death

The cause of death was determined to polydrug use heroin-related deaths due to the combined poisoning of heroin and estazolam.

3.4. Discussion

The dose of heroin that the criminal used was about 1 g, however, the exact purity of that remained unknown. The average street-grade heroin is 2-6% pure and each dosage packet may contain from 13-16 mg of heroin [14]. 415 retail samples of street heroin with a total weight of 128.02 g in Vienna in 1999 were analyzed, containing a median percentage of 6.5% diacetylmorphine (range: 0.0 - 47.0%). All the samples contained a diluent, mainly lactose, as well as adulterants, such as caffeine and/or paracetamol [21]. Heroin profiling is an important practice required for determining indicators of source (such as the geographical origin) or production of the heroin in order to understand drug trafficking organization. We tested the impurities present in the heroin, including diluents and adulterants or production indicators according the articles [6]. Polysaccharides such as starch and monosaccharides such as glucose, lactose, and mannitol are quite often used as dilutions. Adulteration of heroin with pharmacologically active compounds such as paracetamol, phenobarbitone, caffeine, and others are also known [7, 8]. Variable mixture of substances reflects the differences in starting material and the traditional practices of the regions where heroin is produced [17]. Unfortunately, we made an

assignable error in this case that the identification of other ingredients in heroin, including codeine, diluents and adulterants had not been detected. However, the dilutions of heroin such as starch brought about the amorphous yellow substance that showed representative double refracting crystals with the forms of Maltese cross, acicular, rhomb, or irregular in polarizing microscope at the injection site.

Cardinal signs of heroin toxicity mainly include a reduced level of consciousness from drowsiness or stupor to coma, pinpoint pupils and a depressed respiratory rate. Death is usually due to respiratory failure [2013]. The concentration of 6-MAM, morphine and estazolam in blood was 101 ng/mL and 643 ng/mL, 797 ng/mL, respectively, which seems far below the lethal concentrations that reported in the literature: in 7 cases of fatal heroin overdose, postmortem blood concentrations averaged 0.67 mg/L for morphine, 0.78 mg/L for morphine-6-glucuronide and 1.86 mg/L for morphine-3-glucuronide [2]. Importantly, the dominance of the widely held belief that heroin-related fatalities were a consequence of overdose was challenged [10]. Deaths attributed to only heroin appear to form a minority of overdose occasions, concomitant use of other drugs (polydrug use), particularly central nervous system (CNS) depressants such as alcohol and benzodiazepines appeared to be a common. The positive rate of benzodiazepines of heroin overdose samples was 55% in Swedish [12], and 27% in Zador [27]. There was considerable evidence that many instances of overdose were due to the combined effects of opioids with benzodiazepines which were relatively weak respiratory depressants [10], but when combined with morphine they can augment the effects of heroin, increasing the likelihood of a fatal outcome following injection of heroin, due to the potentiation of the respiratory depressant effects of heroin, socalled “polydrug heroin-related deaths” [9, 21,22], which was determined as the cause of death in the decedent. Respiration was controlled principally through medullary respiratory centers with peripheral input from chemoreceptors and other sources. Opioids produce exhibited inhibition via μ and Δ receptors in the medulla. GABA was the major inhibitory neurotransmitters in the control of respiration, which explained the potential for interaction of opioids with benzodiazepines [26]. The deleterious interactions of benzodiazepine and opioid depended on pharmacokinetic or pharmacodynamic mechanisms, relating to additive actions on the different neuromuscular components of the respiration. Certain arguments would support the pharmacodynamic hypothesis: the co-location of GABA and opiate receptors in the central nervous system, the existence of possible cross-reactivity and common pathways of intracellular transduction [19].

Unanimously, the skin tissue showed the maximum concentration of 6-MAM and morphine in the deceased. There are significantly lower heroin peak plasma concentrations after intramuscular injection than intravenous. However, heroin and its metabolites remained in the blood longer after intramuscular compared to intravenous injection, attributing to the sustained heroin release into the circulation [23]. The half-life of heroin and morphine were 2-6min, 2-3 h, respectively, while that of 6-MAM varies from 6 - 25min to 5.4 - 52min [23]. In this case, the interval between the injection of heroin and death was about 45 min, which enabled the complete convertion of heroin into 6-MAM and relative full metabolism of 6-MAM that had been released into circulation, demonstrated by higher blood concentration of morphine (643 ng/mL) than that of 6-MAM (101 ng/mL) and higher concentration of 6-MAM in the liver and kidney compared to blood concentration. The formation of morphine occurred very rapidly, with maximal concentrations measured 3.6 - 8.0 min after heroin administration [23]. Nevertheless, the blood concentration of morphine depended on type of administration, dose, body weight, time elapsed since the last dose and individual pharmacokinetics [2]. It was found that the concentration of morphine in the liver and kidney were almost two times

higher than blood concentration, which was also much higher than 6-MAM blood concentration, indicating that the absorbed morphine in circulation should had reached peak concentration and the level of excretion. The half-life of estazolam ranged from 10-24 h [5] or 8.3-31.2 h (mean 17.0 h) [3]. And the value of elimination half-life did not vary significantly with dose. After single oral doses of 2-16 mg, peak plasma concentrations reached within 6 h. The interval between the intake and death was only about 55 minutes, contributing to a finite absorption of estazolam into circulation and unmatched concentrations of estazolam in the obtained body samples, when take the total dosage of estazolam (144 mg) into consideration.

4. Conclusion

This case analysis provided the important data on the distribution of 6-MAM, morphine and estazolam in human die from combined poisoning of heroin and estazolam. However, further research should be performed for more accurate distribution of combined poisoning of heroin and estazolam. Also, it taught a lesson that the determination of other drugs, particularly central nervous system (CNS) depressants in heroin poisoning were quite important in forensic expertise.

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References

[1] P. H. Abelson, “Death from heroin”, Science, vol. 168, **(1970)**, pp. 1289.
[2] R. Aderjan, S. Hofmann, G. Schmitt and G. Skopp, “Morphine and morphine glucuronides in serum of heroin consumers and in heroin-related deaths determined by HPLC with native fluorescence detection”, Journal Anal Toxicol, vol. 19, **(1995)**, pp. 163-168.
[3] M. D. Allen, D. J. Greenblatt and J. D. Arnold, “Single- and multiple-dose kinetics of estazolam, a triazolo benzodiazepine”, Psychopharmacology (Berl), vol. 66, **(1979)**, pp. 267-274.
[4] R. C. Baselt, “Disposition of Toxic Drugs and Chemicals in Man”, 7th ed. Foster City, CA: Biomedical Publications, **(2004)**, pp. 532-536.
[5] R. C. Baselt, “Disposition of Toxic Drugs and Chemicals in Man”, 7th ed. Foster City, CA: Biomedical Publications, **(2004)**, pp. 409-410.
[6] H. C. Thozet, J. Girard and J. J. David, “Analysis of heroin seized in France”, Bull Narc, no. 44, **(1992)**, pp. 29-33.
[7] R. Coomber, “The adulteration of drugs”, What dealers do, what dealers think. Addict. Res., vol. 5, **(1997)**, pp. 297-306.
[8] R. Coomber, “How often does the adulteration/dilution of heroin actually occur: an analysis of 228 street samples across the UK (1995–1996) and discussion of monitoring”, International Journal Drug Policy, vol. 8, **(1997)**, pp. 178-186.
[9] S. Darke, J. Ross, D. Zador and S. Sunjic, “Heroin-related deaths in New South Wales”, Australia, 1992-1996. Drug Alcohol Depend, no. 60, **(2000)**, pp. 141-150.
[10] I. Demaret, A. Lemaitre and M. Ansseau, “Heroin”, Rev Med Liege, vol. 68, no. 5-6, **(2013)**, pp. 287-93.
[11] C. C. Faura, S. L. Collins, R. A. Moore and H. J. McQuay, “Systematic review of factors affecting the ratios of morphine and its major metabolites”, Pain, no. 74, **(1998)**, pp. 43-53.
[12] A. Fugelstad, “Heroin deaths in Stockholm 1986-1991”, Paper presented at the 5th International Conference on the Reduction of Drug related Harm. Toronto, **(1994)**.
[13] C. R. Gagliano, M. Rutigliano and B. M. Altamura, “Sudden death following ingestion of illicit heroin for concealing”, Acta Med Leg Soc., no. 39, **(1989)**, pp. 453-456.
[14] M. H. Greene, J. L. Luke and R. L. Dupont, “Opiate "overdose" deaths in the District of Columbia. I. Heroin-related fatalities”, Med Ann Dist Columbia, no. 43, **(1974)**, pp. 175-181.

[15] C. E. Inturrisi, M. B. Max, K. M. Foley, M. Schultz, S. U. Shin and R. W. Houde, "The pharmacokinetics of heroin in patients with chronic pain", N Engl J Med., no. 310, **(1984)**, pp. 1213-1217.
[16] B. Jun, Z. Changshu, H. Yi, S. Baohua and Z. Xianyi, "Distinguishing heroin abuse from codeine administration in the urine of Chinese people by UPLC-MS-MS", Journal Anal Toxicol, no. 37, no. 3, **(2013)**, pp. 166-174.
[17] H. Kalant, "Opium revisited: a brief review of its nature, composition, non-medical use and relative risks", Addiction, no. 92, **(1997)**, pp. 267-277.
[18] L. A. King, S. D. McDermott, S. Jickells and A. Negrusz, "Drugs of abuse, in: Jickells S, Negrusz A (Eds), Clarke's analytical forensic toxicology", London, Pharmaceutical Press, **(2008)**, pp. 74-79.
[19] B. Megarbane, P. Gueye and F. Baud, "Interactions between benzodiazepines and opioids", Ann Med Interne (Paris), vol. 154, no. 154, **(2003)**, pp. 64-72.
[20] F. Moriya and Y. Hashimoto, "Distribution of free and conjugated morphine in body fluids and tissues in a fatal heroin overdose: is conjugated morphine stable in postmortem specimens", Journal Forensic Science, no. 42, **(1997)**, pp. 736-740.
[21] D. Risser, A. Uhl, M. Stichenwirth, S. Hönigschnabl, W. Hirz, B. Schneider, C. S. Carion, N. Klupp, W. Vycudilik and G. Bauer, "Quality of heroin and heroin-related deaths from 1987 to 1995 in Vienna", Austria. Addiction, no. 95, **(2000)**, pp. 375-382.
[22] D. Risser, A. Uhl, F. Oberndorfer, S. Hönigschnabl, M. Stichenwirth, R. Hirz and D. Sebald, "Is there a relationship between street heroin purity and drug-related emergencies and/or drug-related deaths? An analysis from Vienna", Austria. Journal Forensic Science, no. 52, **(2007)**, pp. 171-176.
[23] E. J. Rook, A. D. Huitema, W. V. D. Brink, J. M. V. Ree and J. H. Beijnen, "Pharmacokinetics and pharmacokinetic variability of heroin and its metabolites: review of the literature", Curr Clin Pharmacol, no. 1, **(2006)**, pp. 109-118.
[24] A. Y. Salmon, Z. Goren, Y. Avissar and H. Soreq, "Human erythrocyte but not brain acetylcholinesterase hydrolyses heroin to morphine", Clin Exp Pharmacol Physiol, no. 26, **(1999)**, pp. 596-600.
[25] T. A. S. Di, P. Ricci, N. C. Di and P. Cassandro, "A new method for the determination in blood and urine of a novel triazolobenzodiazepine (estazolam) by HPLC", Journal Anal Toxicol, no. 1, **(1986)**, pp. 65-67.
[26] J. M. White and R. J. Irvine, "Mechanisms of fatal opioid overdose", Addiction, no. 94, **(1999)**, pp. 961-972.
[27] D. Zador, S. Sunjic and S. Darke, "Heroin-related deaths in New South Wales, 1992: toxicological findings and circumstances", Med Journal Aust., no. 164, **(1996)**, pp. 204-207.

