

Flunitrazepam in stomach contents may be a good indicator of its massive ingestion

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Abstract: A fatal case involving flunitrazepam with ethanol ingestion is presented. Quantitative toxicological analysis revealed that the concentrations of 7-aminoflunitrazepam (a metabolite of flunitrazepam) and ethanol in a femoral blood sample were 0.175 µg/mL and 0.67 mg/mL, respectively. High concentration of flunitrazepam (24.3 µg/mL) was detected from the stomach contents, and 10.9 mg of flunitrazepam remained in the stomach in total. We concluded that the cause of death was due to massive drug ingestion. A large amount of flunitrazepam in the stomach contents may be a good indicator for massive ingestion because flunitrazepam is rapidly metabolized to its 7-amino metabolite and its bioconversion by bacteria also continues to occur, even in the postmortem period.

Key Words: flunitrazepam, poisoning, stomach contents, massive ingestion.

INTRODUCTION

Flunitrazepam, a 7-nitrobenzodiazepine, is widely prescribed for the treatment of insomnia [1]. It is often abused and observed in combined use with ethanol [2, 3]. Although optimal doses of flunitrazepam are alone generally considered quite safe, fatalities have been reported in cases of overdose or drug interactions with ethanol [3-6].

Forensic diagnosis of poisoning involved flunitrazepam is based on the postmortem blood concentrations of flunitrazepam and its metabolite, 7-aminoflunitrazepam [3-6]. Because the disappearance of flunitrazepam from the blood occurs rapidly by metabolism [4] or postmortem bioconversion [7], the presence of 7-aminoflunitrazepam is an important marker of ingestion of its parent drug, flunitrazepam,

even in a massive overdose case [3, 6, 8].

Here we report a fatal overdose due to flunitrazepam, and we discuss the usefulness of qualitative and quantitative examination of the stomach contents.

CASE REPORT

A Japanese man in his forties (height, 175 cm; weight, 72 kg) was found dead in his room. Autopsy findings indicated no evidence of external injury. An internal examination revealed no distinct injury or disease. The heart weighed 391 g and contained 540 mL of blood with coagulum. The brain weighed 1453 g and was slightly edematous. The left and right lungs weighed 500 g and 606 g, respectively and were moderately congested. The stomach contained a dark brownish fluid (450 mL). Signs other than congestion were not notable in other

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Table 1. Concentrations of flunitrazepam and 7-aminoflunitrazepam in postmortem samples ($\mu\text{g/mL}$)

Specimen	Femoral blood	Urine	Bile	Stomach contents	Therapeutic range*	Lethal range*
Flunitrazepam	0.009	0.010	0.642	24.31	0.005-0.015	
7-Aminoflunitrazepam	0.175	0.082	0.055	0.133		>0.16

*Therapeutic and lethal range is cited from the reference [9, 10]. Lethal range includes total of flunitrazepam and 7-aminoflunitrazepam.

organs. A drug screening test using a TriageTM (Biosite Diagnostic Inc, San Diego, CA) panel was negative. Postmortem blood, urine, and stomach contents samples were collected for toxicological investigation.

Toxicological analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS) was performed according to the manufacturer's instruction. In brief, the liquid chromatography separations were carried out using ekspertTM ultraLC 100-XL (Eksigent part of AB Sciex, Framingham, MA, USA). An L-column2 ODS (1.5 mm \times 150 mm, 5.0 μm particle size, Chemicals Evaluation and Research Institutes, 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, Framingham, MA, USA) was used to obtain the mass spectra. Quantitation of ethanol was performed using headspace gas chromatography.

RESULTS AND DISCUSSION

Toxicological analysis identified flunitrazepam and its metabolite (7-aminoflunitrazepam) in each sample. Table 1 shows the quantification for each substance in the victim's postmortem samples, along with the currently established lethal and therapeutic levels [9, 10]. Ethanol was also quantified from the postmortem samples (0.67 mg/mL in blood and 1.45 mg/mL in urine, respectively).

Although 7-aminoflunitrazepam was well identified, flunitrazepam concentrations in the blood and urine were very low. The presence of 7-aminoflunitrazepam was an important marker of flunitrazepam ingestion [3, 6, 8]. As the sum of flunitrazepam and 7-aminoflunitrazepam concentrations was over 0.16 $\mu\text{g/mL}$ [9], blood levels of flunitrazepam at the time of death reached the fatal range. Based on the autopsy findings and the results of the toxicological examination, we concluded massive ingestion of flunitrazepam combined with ethanol toxicity led to his death.

Since the stomach contents included homogeneous liquid in the present case, we were able to estimate the dose in the stomach contents. A relatively

large amount of unabsorbed drug components (10.9 mg) remained in the stomach. We estimated that 45-73 mg of flunitrazepam had already been absorbed, based on the pharmacokinetic parameters [1]. It has been reported that the amount of residual stomach contents in patients with overdose has no association with the time since ingestion in a clinical study. The amount varies according to the ingested dose, combination of drugs, intake of meals, and other factors [11]. Therefore, it may be difficult to speculate that the victim died shortly after ingestion of a large amount of drug, even with a large amount of drug remaining in the stomach. However, both a high concentration of 7-aminoflunitrazepam in the blood and a large amount of flunitrazepam remaining in the stomach demonstrates massive ingestion, without consideration of the time elapsed since ingestion.

Our results indicate that the victim died following massive oral ingestion of flunitrazepam. In previous reports, no flunitrazepam was detected from the stomach contents in some cases [3, 4] but was found in some other cases [3, 5, 6]. This may be due to various factors such as ingested dose, postmortem interval, environmental circumstances, and presence or absence of combination with alcoholic beverages. A large amount of flunitrazepam in the stomach contents becomes a good indicator of massive ingestion, when it is observed.

As drugs in the stomach contents are uncomplicated by metabolism in general [12], and bioconversion by bacteria is decreased under acidic conditions [7], the parent drug may be detected in its unchanged form [12]. As a large amount of flunitrazepam remained in the stomach, there may have been little effect on its concentration due to postmortem bacterial bioconversion. Therefore, a relatively large amount of flunitrazepam was detected in the stomach contents, despite its easily degradable property [13].

The present case indicates the usefulness of the analysis of stomach contents. It provides various details that are useful in the forensic investigation and diagnosis of overdose cases, and forensic examiners should be more interested in toxicological data from stomach contents.

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

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