Case Report

Toxicokinetics of the Synthetic Cathinone α-Pyrrolidinohexanophenone

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Abstract

Synthetic cathinones inhibit monoamine transporters, such as serotonin, norepinephrine, and dopamine transporters, and act on the central nervous system via increasing synaptic concentrations of monoamines. These compounds, which are highly addictive and potentially poisonous, are new psychoactive substances. In this study, we investigated the toxicokinetics of the synthetic cathinone, α-pyrrolidinohexanophenone (α-PHP), and assessed the relationship between the toxicokinetics and the long-term clinical symptoms induced by α-PHP in a male patient. The patient (39 years old) suddenly started uttering inarticulate words and demonstrating incomprehensible behavior in his house, and was brought to the emergency department of Iwate Medical University hospital. He presented with psychotic symptoms, such as hallucinations and delusion; however, his vital signs were normal. The hallucinations and delusion improved by the third day of hospitalization. Toxicological analysis was performed using liquid chromatography–tandem mass spectrometry with QuEChERS extraction. α-PHP was detected in his serum at a concentration of 175 ng/mL on his arrival at the hospital. His serum concentrations of α-PHP were serially determined and their natural logarithms were plotted against time after arrival. Although serum concentrations at early time points were lacking, the obtained curve was consistent with a two-compartment model and indicated a serum elimination half-life of 37 h. The long-lasting psychotic symptoms induced by synthetic cathinones appear to be correlated with their toxicokinetic characteristics, such as their long half-lives. Finally, interpreting the toxicokinetics of synthetic cathinones may provide useful information for the toxicological assessment of new psychoactive substances for forensic and clinical purposes.

Introduction

Synthetic cathinones are new psychoactive substances that are highly addictive and have the potential to be poisonous. In addition, they are often abused. Synthetic cathinones inhibit monoamine transporters, such as serotonin, norepinephrine, and dopamine transporters, and act on the central nervous system by increasing synaptic concentrations of monoamines. They are classified into three types according to their pharmacological actions: cocaine-3,4-methylenedioxyamphetamine.
mixed-type cathinones, which inhibit the uptake of monoamines in a non-selective manner; methamphetamine-like type cathinones, which preferentially inhibit cathcholamine uptake and release dopamine; and pyrvalerone-type cathinones, which block the selective uptake of catecholamines very potently but do not release the substrate (1).

Clinically, patients with synthetic cathinone poisoning present with psychiatric, neurological, gastrointestinal, cardiovascular and muscular symptoms (2). Moreover, it is well known that synthetic cathinones produce long-lasting toxic effects. We have previously reported that the long-lasting symptoms induced by synthetic cathinones correlate with their long detection window in serum (3). However, the detailed cause of this remains to be elucidated. There have been several reports on the analysis, pharmacology, and metabolites of synthetic cathinones. In addition, there are data on the clinical symptoms and treatment of synthetic cathinone poisoning. However, there are limited data on the toxicokinetics of synthetic cathinones. Understanding the toxicokinetics of these compounds is essential for performing toxicological assessments on new psychoactive substances in clinical and forensic medicine. In the present study, we evaluated the toxicokinetics of α-pyrrolidinohexanophenone (α-PHP), which is an α-pyrrolidinophenone derivative, by measuring its concentration in the serial serum samples of a patient. We also investigated the relationship between the toxicokinetics of α-PHP and the long-term clinical symptoms induced by the compound.

Case History

At 11 pm in April, a 39-year-old male suddenly took a picture through the restroom window of his house screaming, “A ghost is there.” He then ran out of his home speaking incomprehensibly. After several minutes, he was found covered in mud in a rice field owned by his family and was transported to the emergency department of Iwate Medical University hospital. He presented with hallucinations, delusion, fear, anxiety and restlessness. The findings of the clinical examination were as follows: Glasgow coma scale score, 14 (E4V4M6); heart rate, 101 beats/min; blood pressure, 131/68 mmHg; body temperature, 37 ºC; peripheral capillary oxygen saturation (SpO₂), 85% (the SpO₂ improved to 98% after he was administered oxygen); alcohol test, negative; and Triage® drugs of abuse (phencyclidines, benzodiazepines, cocaine metabolite, amphetamines, tetrahydrocannabinol, opiates, barbiturates and tricyclic antidepressants), negative. He was administered an intravenous depressant, negative. He was administered an intravenous administration of 1 mg/mL methanol (high-performance liquid chromatography instrument (Shimadzu, Kyoto, Japan)). The mobile phase was 95% 10 mmol/L ammonium formate: 95% methanol (solvent B). The solvent gradient was increased linearly from 0 to 100% solvent B over 15 min, and maintained for 10 min. The flow rate of the mobile phase was set at 0.1 mL/min. The column temperature was maintained at 40 ºC and the injection volume was 10 µL.

Tandem mass spectrometry (MS-MS) was performed on a 3200QTRAP instrument (AB SCIEX, Framingham, MA, USA). The mass spectrometer was operated in an electrospray ionization positive mode. Quantitative analysis was performed by multiple reaction monitoring in positive ion mode. The precursor ions for α-PHP and IS were at m/z 246.2 and m/z 260.3, respectively, the quantifier product ions for α-PHP and IS were at m/z 91.0 and m/z 105.2, respectively, and the qualifier product ions for α-PHP and IS were at m/z 140.2 and m/z 119.2, respectively.

Sample preparation

α-PHP in serum was extracted according to the QuEChERS method described by Usui et al. (4) with minor modifications. Briefly, a 0.5 mL serum aliquot was diluted with 1 mL of deionized water in a glass tube. The diluted sample was transferred into a plastic tube containing 0.5 g of the pre-packed extraction salts, a stainless steel bead, and 1.0 mL of acetonitrile containing 50 µL of IS (200 ng/mL of MPHP). The mixture was shaken vigorously for 30 s by hand and centrifuged at 2,300 × g for 10 min. The supernatant (600 µL) was transferred into a 2-mL centrifuge tube containing the dispersive solid-phase extraction kit reagent. The tube was mixed by vortexing for 10 s and was then centrifuged at 17,000 × g for 10 min. The upper layer of the mixture (100 µL) was transferred into a glass tube and diluted with 100 µL of deionized water.

Validation of the LC–MS-MS method

The validation design was based on that proposed by Peters et al. (5). Standard solutions of α-PHP were prepared for quality control (QC) and calibrator standards. These solutions (each 1 mg/mL in methanol) were prepared by one researcher on different dates, and were stored at −20 ºC.

QC samples

QC samples were prepared daily at four different concentrations: 1.0 ng/mL, lower limit of quantitation (LLOQ) QC sample; 2.0 ng/mL, low QC sample (LOW); 80 ng/mL, medium QC sample (MED); and 160 ng/mL, high QC sample (HIGH). These QC samples were prepared by spiking blank serum with QC standard solutions of α-PHP.

Selectivity

Blank serum samples from six different, commercially obtained human serum samples were prepared and analyzed to check for peaks that might interfere with the detection of α-PHP and the IS.

Reagents

Standard α-PHP hydrochloride (purity ≥ 98%) and 4′-methyl-α-pyrrolidinohexanophenone (MPHP) hydrochloride (purity ≥ 98%; internal standard (IS)) were purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Methanol (high-performance liquid chromatography (HPLC) grade), acetonitrile (HPLC grade), and ammonium formate were purchased from Kanto Chemical Company Inc. (Tokyo, Japan). A QuEChERS pre-packed extraction pack (containing 6 g magnesium sulfate and 1.5 g sodium acetate) and a dispersive solid-phase extraction kit (containing 25 mg primary secondary amine, 25 mg end-capped octadecylsilane, and 150 mg magnesium sulfate) were purchased from Agilent Technologies (Santa Clara, CA, USA).

Equipment

Liquid chromatography (LC) was performed using an ultra-fast liquid chromatography instrument (Shimadzu, Kyoto, Japan). Chromatographic separation was achieved using an L-column (150 mm × 1.5 mm, i.d.; 5-µm particle size; Chemical Inspection and Testing Institute, Tokyo, Japan). The mobile phase was 95% 10 mmol/L ammonium formate: 5% methanol (solvent A) and 5% 10 mmol/L ammonium formate: 95% methanol (solvent B). The solvent gradient was increased linearly from 0 to 100% solvent B over 15 min, and maintained for 10 min. The flow rate of the mobile phase was set at 0.1 mL/min. The column temperature was maintained at 40 ºC and the injection volume was 10 µL.

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Clinically, patients with synthetic cathinone poisoning present with psychiatric, neurological, gastrointestinal, cardiovascular and muscular symptoms. It is well known that synthetic cathinones produce long-lasting toxic effects. We have previously reported that the long-lasting symptoms induced by synthetic cathinones correlate with their long detection window in serum. However, the detailed cause of this remains to be elucidated. There have been several reports on the analysis, pharmacology, and metabolites of synthetic cathinones. In addition, there are data on the clinical symptoms and treatment of synthetic cathinone poisoning. However, there are limited data on the toxicokinetics of synthetic cathinones. Understanding the toxicokinetics of these compounds is essential for performing toxicological assessments on new psychoactive substances in clinical and forensic medicine. In the present study, we evaluated the toxicokinetics of α-pyrrolidinohexanophenone (α-PHP), which is an α-pyrrolidinophenone derivative, by measuring its concentration in the serial serum samples of a patient. We also investigated the relationship between the toxicokinetics of α-PHP and the long-term clinical symptoms induced by the compound.
addition, a serum sample containing only the IS was analyzed to ensure that MPHP did not interfere with the α-PHP peak.

Calibration, LOD and LLOQ
Replicates (n = 5) of matrix calibration samples at seven different concentrations (1, 2, 5, 10, 50, 100 and 200 ng/mL of α-PHP in serum) were analyzed. These calibrator samples were prepared by spiking blank serum with calibrator standard solutions of α-PHP. Calibration curves were constructed by plotting the peak area ratios of α-PHP to IS versus drug concentration. The curves were then fitted by weighted least squares linear regression with a weighting factor of 1/x. Daily calibration curves were constructed using duplicate measurements per concentration of each validation sample. The limit of detection (LOD) was defined as the quantity of α-PHP in serum that gave a signal-to-noise ratio (S/N) of 3. The LLOQ was defined based on accuracy and precision in addition to the quantity of α-PHP in serum that gave an S/N of 10.

Accuracy and precision
QC samples were analyzed in duplicate daily for 8 days. The concentrations of the QC samples were calculated based on the daily calibration curves. Accuracy was calculated as percentage deviation of the calculated mean concentration at each concentration level from the corresponding nominal concentration. Repeatability and intermediate precision were evaluated as relative standard deviation (RSD) using one-way analysis of variance.

Recovery and matrix effect
Recovery and matrix effect were evaluated using the LOW, MED and HIGH QC samples (n = 7) as follows:

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\text{Recovery(\%)} = \frac{C}{B} \times 100
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\text{Matrix effect(\%)} = \frac{B}{A} \times 100
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A, neat standard solution; B, standard solution spiked after extraction of blank serum; C, standard solution spiked before extraction of blank serum. A, B and C represent absolute peak areas in the concentrations of the respective solutions.

Half-life (T₁/₂) of α-PHP in serum
The natural logarithms of the serum concentrations of α-PHP were plotted against time after arrival at the hospital to obtain a serum concentration–time curve. The time of arrival at the hospital is shown as 0 h. Whole blood samples were collected from the patient at 24, 48, 72, 96, 120, 144, 192, 216, 240, 264, 288, 312, 336 and 360 h after hospital arrival. The whole blood was allowed to clot by leaving it undisturbed at room temperature, and then the clot was removed by centrifuging at 2,000 g for 10 min. These acquired serum samples were stored at −80°C until analysis. Regression analysis of the data was performed using eight concentration–time data points from the terminal natural log-linear phase. The elimination rate constant (kel) was calculated as the slope of the regression line. The elimination serum T₁/₂ of α-PHP was then calculated using the following equation: \(T_{1/2} = 0.693/kel\).

Results and Discussion
No interfering peaks at the retention times of α-PHP and IS were detected in chromatograms for the blank plasma samples. The
chromatogram for the serum sample containing only the IS showed no peak at the retention time of α-PHP (Figure 1). The calibration curves for α-PHP in serum were linear over the concentration range of 1–200 ng/mL. The mean (±standard deviation) slope and intercept, as well as the range of coefficient of determination ($R^2$) values obtained from the daily calibration curves ($n = 8$) that were constructed using duplicate measurements per concentration level were 0.02533 ± 0.000108, 0.00497 ± 0.00086, and 0.9989–0.9998, respectively. The LOD and LLOQ of α-PHP were 0.2 and 1.0 ng/mL, respectively. Repeatability (RSD, %), intermediate precision (RSD, %), and accuracy (bias, %) were, respectively, 5.4, 5.5 and –7.0% for the LLOQ sample; 3.5, 4.5 and –4.4% for LOW; 1.9, 3.6 and 1.7% for MED; and 0.9, 3.4 and –1.5% for HIGH. Recovery and matrix effect were, respectively, 76 and 95% for MED, and 75 and 97% for HIGH. Serum α-PHP concentrations were successfully determined using the LC–MS–MS method. Although a deuterium-labeled analog of the IS was not used in this analysis, the overall precision and accuracy of the method were acceptable.

α-PHP and estimated dihydro-α-PHP (OH-α-PHP) were detected in the patient’s urine on arrival at the hospital by drug screening, which was performed using LC–MS–MS (data not shown); OH-α-PHP is formed by reduction of the β-ketone moiety in α-PHP to the corresponding alcohol metabolite (6). The concentration of α-PHP in the patient’s serum on arrival at the hospital was 175 ng/mL (Figure 2). At 24, 48, 72, 96, 120, 144, 192 and 216 h after arrival at the hospital, the serum α-PHP concentrations were 64.6, 43.6, 27.0, 15.7, 13.4, 6.75, 2.98 and 1.79 ng/mL, respectively (Figure 2). However, only trace amounts of α-PHP were detected in his serum at 240–360 h after arrival at the hospital. As far as we know, there are no reports for comparison on the blood concentrations of α-PHP following α-PHP poisoning. There are reports on blood concentrations of α-pyrrolidinovalerophenone (α-PVP) in intoxication and postmortem cases (3, 7–10). Although α-PHP is a longer-chain homolog of α-PVP, it is difficult to draw meaningful comparisons between the α-PHP concentrations and those of α-PVP. This is because differences in the alkyl chain length from the α-carbon in α-pyrrolidinophenone derivatives affect pharmacological actions, such as the potency of catecholamine transporter blockade (11). However, it appears likely that the concentrations of α-PHP are clinically toxic considering the patient’s clinical presentation.

Serum concentrations of α-PHP in the patient were plotted against time after arrival at the hospital as shown in Figure 2a. To estimate toxicokinetics and calculate the serum elimination $T_{1/2}$ of α-PHP, the natural logarithms of the serum concentrations were plotted against the time after hospital arrival (Figure 2b). Although serum concentrations were lacking at early time points, the curve appears to be consistent with a two-compartment model. The curve in Figure 2b shows good linearity from 24 h after arrival at the hospital: $y = -0.0186x + 4.6428$, $R^2 = 0.9954$. The kel value for α-PHP was 0.0186 h$^{-1}$ and $T_{1/2}$ was 37.3 h. These results suggest that α-PHP is distributed in body tissues, such as the liver, kidney, and brain, similar to its homolog, α-PVP (9, 10). Moreover, it has been reported that the $T_{1/2}$ of the synthetic cathinone, naphthylpyrovalerone, is 34 h (12); however, this $T_{1/2}$ value was estimated from only two points of plasma concentration data. Although the serum elimination $T_{1/2}$ of α-PHP in this study was calculated from a single patient, the $T_{1/2}$ was found to be 37 h, which is consistent with the previously published value. Moreover, α-PHP is thought to be eliminated from the body over a period of 150 h because approximately 94% of the original amount of the agent is eliminated from the body after a period of four times the $T_{1/2}$.

In humans, α-PHP is mainly eliminated in the urine in unaltered and metabolized forms. α-PHP is mainly metabolized in the liver to form OH-α-PHP and 2′-oxo-α-PHP; hydroxylation followed by dehydrogenation on the pyrrolidine ring forms the lactam ring of 2′-oxo-α-PHP (6). This tendency is also observed with other synthetic cathinone α-pyrrolidinophenone derivatives (13). Although we have no renal and hepatic function data for the patient prior to the exposure, it is likely that the $T_{1/2}$ of the synthetic cathinones is affected by renal and/or hepatic dysfunction. In addition, one of the reasons for the long $T_{1/2}$ of α-PHP may be attributed to the gradual release of α-PHP from other body tissues into the blood.

In an in vitro study, 3,4-methylenedioxyxypovalerone (MDPV) demonstrated that high blood brain barrier (BBB) permeability (1). The structure of α-PHP is similar to that of MDPV. In our case, the major adverse effect is psychiatric, and the psychiatric symptoms appear to be consistent with the pharmacological actions, such as inhibiting monoamine transporters in the brain (1). Therefore, it appears that α-PHP easily crosses the BBB and increases the levels of monoamines, such as dopamine and serotonin, in the brain due to its pharmacological action. In addition, α-PHP absorbed into tissues may be released into the blood, followed by continuous absorption into the brain. These findings may be related to the persistent symptoms induced by synthetic cathinones. These long-lasting psychotic symptoms induced by
synthetic cathinones appear to be related to multiple factors, such as the toxicokinetics (e.g., half-life) and pharmacology (e.g., blocking monoamine transporters) of the particular compound.

Overall, in this single patient study, the toxicokinetics of \( \alpha \)-PHP were determined, including a long T\(_{1/2} \) of 37 h. In addition, although there was a lack of serum concentration data at early time points, the pharmacokinetics of \( \alpha \)-PHP is consistent with a two-compartment model. Furthermore, the long-lasting psychotic symptoms induced by the synthetic cathinone may be linked to its toxicokinetics. Our data on the toxicokinetics of \( \alpha \)-PHP may be useful in the toxicological assessment of new psychoactive substances.

References