Simultaneous Measurement of Urinary Ketamine, Norketamine, and Dehydronorketamine by Liquid Chromatography-atmospheric Pressure Chemical Ionization Mass Spectrometry

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A liquid chromatography/atmospheric pressure ionization mass spectrometry has been developed for the determination of ketamine, norketamine, and dehydronorketamine in human urine. A separation of these analytes in urine samples without tedious pretreatment procedures has been achieved within 10 min. Linear calibration curves of these analytes with coefficients better than 0.998 have been obtained over a wide range from 12.5 to 200 ng/mL. The accuracy was between 2.1% and 7.2% with detection limits at levels of 0.02 ng/mL, 0.02 ng/mL and 0.93 ng/mL for ketamine, norketamine and dehydronorketamine, respectively. The results demonstrate the suitability of the liquid chromatography/atmospheric pressure ionization mass spectrometry approach to analyze trace ketamine, norketamine and dehydronorketamine in urine. Urinary ketamine and norketamine levels were relatively low at 4-24 h intervals and were difficult to assay in a normal laboratory. In the present study, the determination of urinary dehydronorketamine levels at 2-24 hours appears to have a great potential for use in Schedule III controlled drugs management.

Keywords: APCI; Dehydronorketamine; LC; Ketamine; MS; Norketamine; Urine.

INTRODUCTION

Ketamine is a commonly used short-acting anesthetic agent with non-competitive N-Methyl-D-aspartate (NMDA) receptor antagonist activity. It has been reported that ketamine produced analgesic effects for neuropathic pain of tolerance in a low dose without anesthetic. Ketamine may be taken orally to produce an effect similar to that of phencyclidine (PCP) or lysergic acid diethylamide (LSD). A long-term user could have drug resistance and dependence. The Drug Enforcement Administration (DEA) of the United States and the National Bureau of controlled Drugs (NBCD) of Taiwan have classified ketamine as a Schedule III Controlled Substance.

Ketamine is mainly metabolized in the liver via the enzyme P-450 to norketamine with a half-life of approximately two hours. Norketamine also has an anesthetic effect with approximately 1/3 the potency of ketamine and may contribute to the analgesic effects following ketamine administration. Norketamine can be further oxidatively metabolized to form dehydronorketamine which is highly water-soluble. Several analytical methods have been developed for the determination of ketamine, norketamine and dehydronorketamine in plasma, urine and the brain with GC, LC and MS. However, the methods described require derivatization or column extraction, or they lack sensitivity for accurate determination of ketamine and/or its metabolites simultaneously.

The atmospheric pressure ionization (API) techniques have significantly increased the availability of mass spectrometry as a chromatographic detector. The use of hyphenated API-MS techniques for the detection of pharmaceutical compounds is known to afford high sensitivity. In the present study, LC with atmospheric pressure chemical
ionization (APCI) technique was developed to simultaneously determine ketamine, norketamine and dehydronorketamine in human urine samples.

METHODS AND MATERIALS

Chemicals and reagents
Ketamine solution (50 mg/mL) was purchased from Parke-Davis (Parke-Davis Company, Taiwan). Norketamine and dehydronorketamine were generously supplied by Pfizer (Warner-Lambert Company, Ann Arbor, MI, USA). All solutions were prepared using HPLC-grade acetonitrile and deionized water throughout. Ammonium acetate and acetic acid were purchased from Aldrich (Aldrich, Chem. Co. Milw., WI, USA). All chemicals were used without further purification. Aqueous stock solutions of ketamine, norketamine and dehydronorketamine were prepared and stored at 4 °C. Aqueous working solutions of ketamine, norketamine and dehydronorketamine were also stored at 4 °C.

Urine sample collection
Thirty volunteers (mean age, 31 years; 15 males and 15 females) provided informed consent (IRB SK#9012-001). Ketamine solution (Parke-Davis Company, Taiwan) was injected into each volunteer (10 mg/per person, i.m.). Urine samples (5 mL) were collected from each volunteer at 1 h, 2 h, 4 h, and 24 h after ketamine administration. Urine samples were collected, filtered (0.2 μm Nylon syringe filter) and analyzed directly without pretreatment, or were filtered into a collection tube and stored at -20 °C until analysis. The study protocol was approved by the Shin-Kong Wu Ho-Su Memorial Hospital Ethics Committee on Research and National Bureau of Controlled Drugs (9012-001).

LC-APCI-MS conditions
LC-APCI-MS analysis was carried out using a Surveyor MS pump (Thermal Quest, San Jose, CA, U.S.A) linked to a Finnigan LCQ mass spectrometer (Thermo Finnigan, San Jose, CA, USA). The MS was equipped with an atmospheric pressure chemical ionization (APCI) interface. Aliquots of a urine sample (5 μL) were assayed in the LC-APCI-MS system. Separation of these analytes was achieved using a Supelcosil LC-18DB column (4.6 x 250 mm, 5 μm, Supelco). The mobile phase consisted of acetonitrile and 0.03 M ammonium acetate buffer (50:50, v/v) and was adjusted to pH 7.1 by conc. acetic acid. The mobile phase was filtered through a 0.22 μm Nylon filter under reduced pressure and helium-degassed for 20 min. The flow rate was set at 1.0 mL/min.

The mass spectrometer was tuned using a standard solution containing ketamine, norketamine, and dehydronorketamine (100 ng/mL), and the optimized tune and parameters were as follows: capillary temperature of 160 °C, spray voltage at 6.5 kV, sheath gas flow at 1.35 L/min, auxiliary gas flow at 6 L/min and capillary voltage at 45 V. Optimized lens values were: tube lens offset at -15 V, octapole RF Amplitude (Vp-p) at 400, octapole 1 offset (V) at -0.25, octapole 2 offset (V) at -5.50 and interoctapole lens at -15 V. The standard Finnigan LCQ software package was used for instrumental control, data acquisition and processing.

RESULTS AND DISCUSSION
A typical total ion chromatogram of a spiked urine sample containing ketamine, norketamine and dehydronorketamine (100 ng/mL each) is shown in Fig. 1. The separation of ketamine, norketamine and dehydronorketamine was achieved with retention times of 5.0, 5.5 and 7.4 min, respectively.

Under the APCI-MS mode, typical mass spectrum of a spiked urine sample containing (A) ketamine (B) norketamine and (C) dehydronorketamine (100 ng/mL each) is shown in Fig. 2. The characteristics of pseudomolecular ions (M+H+) of each analyte (ketamine at m/z = 238; norketamine at m/z = 224; and dehydronorketamine at m/z...
were used for LC-APCI-MS selective ion monitoring.

Calibration curves of ketamine, norketamine and dehydronorketamine were constructed by plotting pseudomolecular ion peak areas vs. concentrations of each analyte. They were linear in the range of 12.5-200 ng/mL ($R^2$ were between 0.998 and 0.999) with limits of detection between 0.02-0.93 ng/mL shown in Table 1. The precisions ($n = 6$) of the LC-APCI-MS assay in urine samples supplemented with ketamine, norketamine and dehydronorketamine (25 ng/mL each) were 3.2, 2.1 and 7.2%, respectively, as shown in Table 1.

The method was applied in the determination of ketamine, norketamine and dehydronorketamine concentrations in urine samples of thirty volunteers after i.m. injection of ketamine at a dose of 10 mg/per person. Typical mass ion chromatograms produced by LC-APCI-MS containing (1) dehydronorketamine (2) norketamine and (3) ketamine of urine samples after 10 mg ketamine (i.m.) administrated at (A) 1 hour (B) 2 hours (C) 4 hours and (D) 24 hours are shown in Fig. 3.

Differences in levels of urine ketamine and its metabolites may be explained by individual variations in accumulation and/or metabolism after a single administration of ketamine. The time courses of mean concentrations of ketamine, norketamine and dehydronorketamine in urine are shown in Fig. 4.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Linear range (ng/mL)</th>
<th>$R^2$</th>
<th>L.O.D. (ng/mL)</th>
<th>R.S.D. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine</td>
<td>12.5-200</td>
<td>0.998</td>
<td>0.93</td>
<td>3.2</td>
</tr>
<tr>
<td>Norketamine</td>
<td>12.5-200</td>
<td>0.999</td>
<td>0.02</td>
<td>2.1</td>
</tr>
<tr>
<td>Dehydronorketamine</td>
<td>12.5-200</td>
<td>0.999</td>
<td>0.02</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Fig. 2. Typical mass spectra of (A) ketamine, (B) norketamine, and (C) dehydronorketamine from a spiked urine sample were analyzed by LC-APCI-MS.

Fig. 3. Mass ion chromatograms produced by LC-APCI-MS containing (1) dehydronorketamine (2) norketamine and (3) ketamine of a volunteer’s urine samples after 10 mg ketamine (i.m.) administrated at (A) 1 hour (B) 2 hours (C) 4 hours and (D) 24 hours.
Ketamine concentrations were detected in 29 out of 30 urine samples 1 hour after injection with a mean of 25.8 ng/mL. Ketamine concentrations could be detected in all volunteers’ urine samples 2 hours after injection with a mean of 14.4 ng/mL. They were detected in 27 out of 30 volunteers’ urine samples 4 hours after injection with an average of 8.0 ng/mL. However, they were detected in only 13 out of 30 volunteers’ urine samples 24 hours after injection with very low values between 1.4 and 7.5 ng/mL (the average concentration was 1.5 ng/mL). The maximum ketamine concentrations were observed at 1 hour after administration. Thereafter, urinary ketamine levels decreased over time and could still be detected at 24 hours after administration in one-third of the volunteers’ urine samples.

Norketamine concentrations were also detected in 29 out of 30 urine samples 1 hour after injection with an average concentration of 10.6 ng/mL. Norketamine concentrations increased and could be detected in all urine samples 2 hours and 4 hours after injection with average concentrations of 21.4 and 13.1 ng/mL, respectively. They were detected in 28 out of 30 volunteers’ urine samples 24 hours after injection with values between 1.1 and 12.9 ng/mL (average was 4.1 ng/mL).

Dehydronorketamine concentrations were detected in 29 out of 30 volunteers urine samples 1 hour after injection with an average concentration of 26.5 ng/mL. Dehydronorketamine concentrations could be detected in all volunteers’ urine samples at 2 hours and 4 hours after administration with average concentrations of 85.2 and 72.2 ng/mL, respectively. These levels were much higher than levels observed at 1 hour. In addition, it could be detected in all volunteers’ urine samples at 24 hours after administration with an average concentration of 26.0 ng/mL. It is important to develop rapid and accurate methods to determine drugs and their metabolites in biological samples. The abuse of ketamine has gained popularity in recent years.

In general, the metabolites of ketamine are norketamine and dehydronorketamine in blood, and urine. Adams et al. previously reported that dehydronorketamine is a methodological artifact resulting from thermal dehydration of norketamine after liquid-liquid extraction. However, Bolze and Boulieu verified that dehydronorketamine is not due to transformation. It is a metabolite recovered in human plasma in significant concentrations. Our data further supports previous findings that dehydronorketamine is a major metabolite in human urine.

In conclusion, the present assay is a reliable and simple procedure which yields linear standard curves over the urinary concentration range studied. The intra-assay precision is high with sensitive limits of detection making the assay suitable for human urine samples up to 24 hours after administration of ketamine. The method described is simple, selective and very sensitive, which makes it a suitable tool for monitoring urinary ketamine and its metabolites simultaneously after administration of a low dose of ketamine. With regard to Schedule III controlled drugs management, urinary ketamine and norketamine levels were relatively low at 4-24 h intervals. They may be difficult to assay in a normal laboratory. However, a shorter interval may be a better alternative for controlled drugs management as the urinary dehydronorketamine levels were relatively higher at 2-24 hours.

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