K22 Elimination of Ketamine and Norketamine in Urine of Nonhuman Primates After a Single Dose of Ketamine

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Upon completion of this presentation, the attendee will understand the principles of extraction and detection of ketamine (KET) and norketamine (NKET) in urine using NCI-GC-MS, 2) concentrations of KET and NKET in nonhuman primate urine after a single dose of the drug.

The general anesthetic ketamine (Ketalar®, Ketaject, Vetalar) (KET) is used in human and veterinary medicine for induction of anesthesia for short surgical procedures and routine veterinary procedures. It has also been identified as a so-called "date-rape" drug for the purpose of "drugging" unsuspected victims and raping them while under the influence of the drug. Its illicit use by teenagers in rave parties has also been reported. The objective of this paper was to study elimination of KET and its major metabolite norketamine (NKET) in urine collected from five nonhuman primates which received a single dose of KET, and to study elimination patterns to determine how long after drug administration, KET and metabolite can be detected. The data are of great importance to law enforcement agencies and the forensic toxicology community in order to determine how long after sexual assault the urine samples can be collected from the victim to successfully prosecute the perpetrator. The aim of this study was: 1) to develop and validate highly sensitive NCI-GC-MS method for the simultaneous quantitation of KET and its major metabolite NKET in urine, 2) to analyze urine samples collected from nonhuman primates which received a single dose of KET, for NKET and KET.

Method: Urine was collected from five stumptail macaques (*Macaca arctoides*), four females (8-19 kg) and one male (17 kg) caged individually. All animals received a wash-out period of six months prior to the experiment. One urine sample was collected from each animal before KET administration. All monkeys received a single dose (5 mg/kg, IM) of KET. Urine samples were collected from each animal for 18 hours every day (excluding weekends) up to 24 days and once every four days up to 35 days.

Extraction: All urine samples (2 ml) were extracted from urine using HCX solid phase extraction columns. Five point standard curves for KET and NKET were prepared by spiking aliquots (2 ml) of negative urine. The range of the standard curves was 20-1,000 ng/ml for KET and 50-5,000 pg/ml for NKET. In addition, two levels of control urine preparations were analyzed (100 pg/ml and 1,200 pg/ml for NKET, and 40 ng/ml and 750 ng/ml for KET). To all standard, control and study samples, internal standards (D_4 NKET 1,000 pg/ml), 0.1 M acetate buffer (pH 4.5, 1 ml) and crude β-glucuronidase solution (50 ml) were added, and samples were incubated for 1.5 hours at 37°C. After incubation 1.93 M acetic acid (1 ml) and deionized water (10 ml) were added. An analytical column was conditioned with methanol (3 ml) deionized water (3 ml) and 1.93 M acetic acid (1 ml), the sample was added and the column was washed with deionized water (3 ml), 0.1 N HCl (1 ml) and methanol (3 ml). The final elution from the extraction column was achieved using methylene chloride:isopropanol:ammonia (78:20:2:, v/v/v, 3 ml). All extracts were evaporated to dryness in the stream of nitrogen, dissolved in ethyl acetate (50 ml) and transferred to autosampler vials. Dried samples were derivatized (30 min, 60°C) using HFBA (50 ml). HFBA was evaporated under vacuum and the dry residue was dissolved in ethyl acetate (25 ml).

Analytical Procedure: A Hewlett-Packard GC-MS instrument (6890 GC and 5973 MSD) operating in chemical ionization mode was used for the analysis. The column was an HP5-MS (30 m length x 0.2 mm i.d. x 0.25 mm film thickness) and the collision gas was methane maintained at an ion gauge pressure of 3.9×10^{-4} Torr. The injector temperature was 240°C, the transfer line was 280°C and the source and quadrupole were kept at 200°C and 106°C, respectively. The oven was held at 60°C for 1 min then ramped at 30°C/min to a final temperature of 310° where it was held for 3 min. The injection volume was 1 ml. The monitored ions for KET derivative were *m/z* 226 and 357, for NKET *m/z* 383 and 399, and for D_4 NKET *m/z* 387 and 403.

Results: In two monkeys KET was detected in urine up to three days after drug administration (7,070-32 ng/ml), in one up to four days (13,500-65 ng/ml), in one only on day 1 and 2 (4,000 and 70 ng/ml, respectively), and in one animal ten days after KET injection (35,000-22 ng/ml). NKET concentrations in urine ranged from 1.75 mg/ml to 63 pg/ml and it remained in urine throughout the entire 35-day study period in four out of five animals. In one monkey NKET was detected up to 31 days after KET administration.

Date-Rape Drugs, Ketamine and Norketamine, Urine, NCI-GC-MS

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