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Ecotoxicological effect of ketamine: Evidence of acute, chronic and photolysis toxicity *to Daphnia magna*

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ABSTRACT

Ketamine has been increasingly used in medicine and has the potential for abuse or illicit use around the world. Ketamine cannot be removed by conventional wastewater treatment plants. Although ketamine and its metabolite norketamine have been detected to a significant degree in effluents and aquatic environments, their ecotoxicity effects in aquatic organisms remain undefined. In this study, we investigated the acute toxicity of ketamine and its metabolite, along with the chronic reproductive toxicity of ketamine (5–100 μg/L) to *Daphnia magna*. Multiple environmental scenarios were also evaluated, including drug mixtures and sunlight irradiation toxicity. Ketamine and norketamine caused acute toxicity to *D. magna*, with half lethal concentration (LC_{50}) values of 30.93 and 25.35 mg/L, respectively, after 48 h of exposure. Irradiated solutions of ketamine (20 mg/L) significantly increased the mortality of *D. magna*; pre-irradiation durations up to 2 h rapidly increased the death rate to 100%. A new photolysis byproduct (M.W. 241) of norketamine that accumulates during irradiation was identified for the first time. The relevant environmental concentration of ketamine produced significant reproductive toxicity effects in *D. magna*, as revealed by the reduction of the number of total live offspring by 33.6–49.8% ($p < 0.05$). The toxicity results indicate that the environmental hazardous risks of the relevant ketamine concentration cannot be ignored and warrant further examination.

1. Introduction

Pharmaceuticals are detected frequently in aquatic environments; hospital usage, drug abuse and household wastewater discharge are major contributors to the aquatic environment and gradually threaten ecosystems. Neuroactive drugs, such as narcotics, amphetamines, illicit substances, and non-narcotic drugs, have a high potential for abuse or illicit use (Zuccato et al., 2008). Among these drugs, the use of ketamine, an anesthetic and analgesic agent primarily used in veterinary medicine and pediatrics, is increasing among the general population because it confers a hallucinogenic “high” state or a “rave wave” in nightclubs and dance clubs at super-clinical doses (Morgan and Curran, 2012; Murray, 1998). Currently, the popularity of ketamine has increased rapidly within the adolescent drug culture around the world, especially in Asia (Cheung and Ch'ien, 1996; Lian et al., 2005; Liu et al., 2005; Noorzurani et al., 2010). Previous reports indicated that ketamine cannot be removed completely by natural purification processes (such as sorption, photolysis, hydrolysis, volatilization and biodegradation) or by conventional wastewater treatment plants (Baker and Kasprzyk-Hordern, 2013; Lin et al., 2010, 2014). Thus, the residuals remain chemically and biologically active in the aquatic environment. High concentrations of ketamine (from 0.1 μg/L to 10 μg/L) have been detected in effluent samples (Baker and Kasprzyk-Hordern, 2013; Du et al., 2015; Lin et al., 2014), and ketamine is frequently and persistently detected in the receiving surface waters (Li et al., 2016; Lin et al., 2014). In addition, a study has demonstrated that a mass event, such as a music festival, can result in a significant release of ketamine into the environment; up to 9.53 μg/L and 138 μg/L of ketamine were detected in surface water and wastewaters, respectively (Jiang et al., 2014).

Many pharmaceuticals have been reported to cause ecotoxicity, and the acute toxicity to *Daphnia magna* has been the focus of previous studies. The complete and detailed toxicity data are summarized in the Supplementary material (See Table S2). The acute toxicity values (immobilization or mortality within 48 h and the half maximum effective concentration or lethal concentration, EC_{50}/LC_{50}, value) of pharmaceuticals to *D. magna* have been generally investigated in the mg/L range. For example, Ferrari et al. (2003) demonstrated that the acute toxicity values (48-h immobilization, EC_{50} value) of diclofenac, clofibric acid and carbamazepine to *D. magna* were 224.3, > 13.8 and > 200 mg/L, respectively. A similar toxicity behavior was also observed in nervous system drugs; substances such as amphetamine

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sulfate, caffeine, diazepam, clofibrinic acid, phenobarbital, diphenylhydantoin and carbamazepine were reported to exhibit an acute toxicity level above the mg/L level (Cleuvers, 2003; Ferrari et al., 2003; Kim et al., 2007; Lilius et al., 1995).

In terms of chronic toxicity, individual pharmaceuticals (such as antibiotics, antibacterial drugs, anti-inflammatory drugs and nonsteroidal anti-inflammatory drugs) induce reproductive toxicity in *D. magna* primarily at the mg/L level (Carlsson et al., 2006; Cleuvers, 2008; Stuer-Lauridsen et al., 2000; Wollenberger et al., 2000). However, at low or environmentally relevant concentrations (ng/L–μg/L), different effects on the reproductive system have been observed. Some of the existing literature has shown that most pharmaceuticals exhibit no obvious reproductive effects at low concentrations (Carlsson et al., 2006; Köpf, 1995; Schweinfurth et al., 1996; Stuer-Lauridsen et al., 2000; Wollenberger et al., 2000). Meinertz et al. (2010) reported that diphenhydramine hydrochloride and erythromycin thiocyanate do not significantly impact *D. magna* survival and reproduction at 5 times (0.023 μg/L) and 40 times (6 μg/L) the maximum reported environmental concentration for 21 days in a continuous exposure test system. However, in a few cases, studies have also demonstrated that neuroactive drugs result in increased offspring reproduction. For example, fluoxetine and carbamazepine have significantly produced more offspring at 80 and 1 μg/L, respectively, than those in controls in *D. magna* (Campos et al., 2012; Lürling et al., 2006). Rivetti et al. (2016) demonstrated that neuro-active pharmaceuticals (diazepam and carbamazepine) could enhance reproduction in *D. magna* at low environmental levels (0.1 μg/L of diazepam and 1 μg/L of carbamazepine).

The acute and chronic toxicity of ketamine in aquatic organisms is currently unknown. The only three existing studies are as follows. Fick et al. (2010) used a model to calculate and predict that ketamine up to 40 μg/L will cause a pharmacological effect in fish. A toxicity assessment report (Liao et al., 2015) indicated that ketamine (0.95–9500 μg/L) and methamphetamine (0.60–6000 μg/L) delayed blood circulation and hatching time in embryos of medaka fish and altered larval alteration in early life stages. In addition, Lin et al. (2014) demonstrated that even though phototransformation, one of the natural attenuation processes, significantly reduces the concentration of ketamine and it's metabolite norketamine in aquatic systems, the solutions of ketamine and norketamine exhibit higher Microtox® toxicity after 14–20 h of sunlight exposure.

The presence of ketamine has always been accompanied by the presence of norketamine in the aquatic environment. The ketamine to norketamine ratio (K/NK) was found to be 0.7 – 4.6 in hospital effluents and 0.3–0.9 in receiving river waters in Taiwan (Lin et al., 2014). This ratio is close to that found in human urine, with a mean ratio of 0.743 (range of 0.13–2.55) reported in the USA (Moore et al., 2001) and 1.76 (range of 0.49–3.56) in Korea (Kim et al., 2008). Similarly, pharmaceuticals do not always contain only one compound; cocktails of drug mixtures have been demonstrated to have a greater impact on environmental organisms compared to that of a single compound. For example, a mixture of 5 estrogenic chemicals induce additive effects on the reproductive performance (fathead minnows) at environmentally relevant concentrations (in the μg/L range) with increasing levels of biological complexity (Brian et al., 2005, 2007). Our recent study (Li and Lin, 2015) tested mixtures of 19 compounds (including antibiotics, β-blockers, lipid regulators, psychiatric drugs and nonsteroidal anti-inflammatory drugs) on *Cyprinus carpio* and demonstrated that the mixtures induced a synergistic increase in mortality. The toxicity of a pharmaceutical mixture of β-blockers and nonsteroidal anti-inflammatory drugs (NSAIDs) has been modeled using QSAR in previous studies (Altenburger et al., 2003; Escher et al., 2006).

To the best of our knowledge, this is the first study on the acute and chronic toxicity of ketamine and its metabolite norketamine in *D. magna* and the first work to investigate the photolysis and synergistic aquatic toxicity of ketamine/norketamine (K/NK) in aquatic organisms. Scenarios with different K/NK ratios (including the ratios of hospital effluents, receiving river waters and urine samples) were simulated and evaluated. One new phototransformation byproduct was identified for norketamine, and the chronic exposure amount (μg/L) of ketamine and its byproducts at environmentally relevant concentrations was evaluated.

2. Materials and methods

2.1. Daphnia magna

The culture method was performed primarily according to our previous study (Li and Lin, 2015) and the standard methods NIEA B901 (*Daphnia magna*) of the Taiwan Environmental Protection Agency. The *D. magna* cultures consisted of 4-L glass beakers containing 3 L of culture medium; the beakers were housed in an environmental chamber. The culture medium was renewed and the produced offspring were discarded weekly. Brood daphnids were discarded after 4 weeks in culture and replaced with neonatal organisms. For the cultures, the temperature in the chamber was controlled at 25 ± 2 °C, the photoperiod was 16 h of light and 8 h of darkness, and the cultured daphnids were fed daily. In addition, the reference toxicity test was conducted during the toxicity test, and the results were used to evaluate the maintenance and quality assurance of the toxicity laboratory. Sodium chloride was chosen as the reference toxicant in this study.

2.2. Chemicals and standards

LC-grade methanol was obtained from Mallinckrodt Baker (Phillipsburg, PA, USA). ACS-grade formic acid was purchased from Riedel-deHaen (Seelze, Germany). Ketamine hydrochloride (100%), magnesium sulfate heptahydrate (> 99.5%), sodium bicarbonate (> 99%), sodium hydroxide and sulfuric acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Norketamine hydrochloride (> 99%) was purchased from Tocris Bioscience (Bristol, UK). Calcium sulfate dihydrate (> 98%) and potassium chloride (> 99%) were purchased from J. T. Baker (Center Valley, PA, USA). Individual stock standard solutions were prepared on a weight basis in DI water. These solutions were stored in amber glass bottles at 4 °C for a maximum of 30 days.

2.3. Chemical analysis of the pharmaceuticals

The chemical analytical quantification methods used in this study followed those described in our previous work (Lin et al., 2014). Ketamine, norketamine and the byproducts were chromatographically separated using an Agilent 1200 module (Agilent, Palo Alto, CA, USA) equipped with a ZORBAX Eclipse XDB-C18 column (Agilent, Palo Alto, CA, USA, 150 × 4.6 mm, 5 μm). A binary gradient with a flow rate of 1.0 mL/min was used. Mobile phase A contained 0.1% formic acid (v/v) in water. Mobile phase B contained 0.1% formic acid (v/v) in methanol.

The mass spectrometric measurements were performed on a Sciex API 4000 (Applied Biosystems, Foster City, CA, USA) equipped with an electrospray ionization (ESI) interface. The ketamine and norketamine analyses were performed in positive mode and multireaction monitoring (MRM) with the following ion transitions: ketamine *m/z* 238/125 (and for confirmatory purposes *m/z* 238/220) and norketamine *m/z* 224/125 (and for confirmatory purposes *m/z* 224/179). The detailed gradients and mass spectrometer conditions used are available in our previous work (Lin et al., 2014).

Ketamine and norketamine were investigated separately at high initial concentrations (20 mg/L) for the byproduct investigation. Full-scan mode was used to detect the byproducts in the degradation mixture and to obtain their mass spectra. The signal areas of the byproducts were quantified using LC-MS/MS. An ACD MS fragmenter (Advanced Chemical Development, Toronto, ON, Canada) was used to generate a fragmentation tree for the ketamine or norketamine