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Ketamine Intoxication in Rats

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This report provides preliminary information on overt toxicity and incapacitation following intraperitoneal injection of ketamine in rats. The ketamine doses were 37.5, 75.0, and 150.0 mg/kg, and selected to be below the lethal range but potentially intoxicating. Rapid immobilization was observed at the two highest doses, whereas the lower dose produced only transient ataxia and light sedation. Behavioral performance was indexed by the acquisition of a two-way discriminated active shuttle avoidance response 24 hours after ketamine injection. This assessment revealed no apparent differences from vehicle control performance, suggesting complete to near complete functional recovery by this time. Overnight food consumption revealed no robust or lingering effects of ketamine injection, but weight loss was observed at the highest ketamine dose. These data and their conclusions are limited by the small number of subjects, limited ranges of doses, and inclusion of only one exposure route. Nevertheless, this study provides important preliminary information on the rapid onset and relatively short duration of intoxication that can be achieved by ketamine in a rat model. Utilizing this same animal model, more prompt and comprehensive operant behavioral testing following ketamine exposure appears warranted to more extensively characterize this potent incapacitating drug and for discovering effective medical countermeasures for ketamine intoxication.

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ABSTRACT

This report provides preliminary information on overt toxicity and incapacitation following intraperitoneal injection of ketamine in rats. The ketamine doses were 37.5, 75.0, and 150.0 mg/kg, and selected to be below the lethal range but potentially intoxicating. Rapid immobilization was observed at the two highest doses, whereas the lower dose produced only transient ataxia and light sedation. Behavioral performance was indexed by the acquisition of a two-way discriminated active shuttle avoidance response 24 hours after ketamine injection. This assessment revealed no apparent differences from vehicle control performance, suggesting complete to near complete functional recovery by this time. Overnight food consumption revealed no robust or lingering effects of ketamine injection, but weight loss was observed at the highest ketamine dose. These data and their conclusions are limited by the small number of subjects, limited ranges of doses, and inclusion of only one exposure route. Nevertheless, this study provides important preliminary information on the rapid onset and relatively short duration of intoxication that can be achieved by ketamine in a rat model. Utilizing this same animal model, more prompt and comprehensive operant behavioral testing following ketamine exposure appears warranted to more extensively characterize this potent incapacitating drug and for discovering effective medical countermeasures for ketamine intoxication.

INTRODUCTION

Ketamine (KET; Cl-581) is an arylcyclohexylamine derivative developed on the backbone of phenyl-cyclohexyl-piperidine (phencyclidine; commonly known as PCP) as an alternative to the then-popular anesthetic with a lower incidence of psychotomimetic side effects. KET is classified as a dissociative anesthetic, as it produces a functional dissociation between the limbic and thalamocortical systems. While notably an N-methyl-D-aspartate (NMDA) receptor antagonist, KET has been shown to be active at a wide variety of receptors and receptor subtypes including, but not limited to, μ, δ, and κ opioid receptors, sigma receptors, and nicotinic and muscarinic cholinergic receptors, as well as to inhibit serotonin uptake and interact with acetylcholinesterase [1-6]. An active metabolite of KET, (2R,6R)-hydroxynorketamine, has also been shown to activate α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors [7]. KET, in its hydrochloride salt form, is highly water-soluble and can be administered intravenously, intramuscularly, subcutaneously, rectally, sublingually, orally, transdermally and intranasally, as well as through a nebulized aerosol solution [8-11]. KET is rapidly and widely distributed following intravenous administration and follows a two-compartment model of elimination, with α and β phase half-lives of 11-17 minutes and 151-186, respectively [12]. KET is typically found in the form of a racemate hydrochloride salt, with both the R(-) and S(+) optical isomers present in equal amounts. In human subjects, the S(+) enantiomer induces more EEG suppression than the R(-) equivalent dose, with the R(-) enantiomer being unable to achieve the same level of EEG suppression as S(+) at higher doses due to a ceiling effect [13]. Similarly, the S(+) enantiomer has considerably more potency as a hypnotic and analgesic, as well as when inducing anesthesia (which R(-) often fails to achieve altogether) [14]. Recovery from the anesthetic effects of KET is shortest when S(+) is used alone rather than when R(-) or the racemate is used, suggesting an additive effect between the two enantiomers when administered together, possibly due to R(-) inhibiting the elimination of the more active S(+) enantiomer [13, 15]. The only clinical benefit of R(-) KET when used alone is that it may be a more potent and long-lasting anti-depressant, perhaps owing to its reduced psychotomimetic effects [16]. KET has most often been studied in its racemic form, as is the case in the present study.

KET's use in humans dates back to the mid-1960's when it was shown to be an effective fast- and short-acting anesthetic when given intravenously [17]. More recently it has been shown to have both analgesic [2, 18] and antidepressant [7, 19] effects at sub-anesthetic blood concentrations, which has broadened its use in clinical settings. Further, it has been shown to be an effective drug adjunct for treating numerous types of status epilepticus (SE), including refractory SE [20-23]. In some areas, KET is being utilized at supra-anesthetic doses for the rapid and prolonged sedation of acutely agitated patients in emergency medical settings via large-volume intramuscular injection [24, 25]. KET has also been shown to be a safe and effective sole anesthetic agent in human clinical settings across a wide variety of doses during major surgical procedures such as open-heart surgeries, open-chest pulmonary surgeries, long-bone fracture repairs, and skin grafts [26-29]. In most cases of KET being used as a sole anesthetic agent in humans, pre- or co-medication with drugs such as atropine, diazepam, and various paralytic agents was used. Fatal KET overdoses, while rare due to KET's high therapeutic index, mostly occur in the context of recreational drug abuse and often involve other factors such as underlying illness or injury and poly-drug abuse [30-33].

KET has been evaluated for use as a safe and rapid incapacitating agent in numerous large animal species (such as lions, chimpanzees, orangutans, horses, badgers, giraffes and otters, among others), both as a sole agent [34-36] and in combination with other drugs, such as the phenothiazine drug promazine, the opioid butorphanol, and the α2-adrenergic antagonists xylazine and medetomidine (including its various derivatives) [37-42]. When used as a sole incapacitating agent in badgers, KET

doses ranged between 9.5 and 31.0 mg/kg intramuscularly (including repeated dosing), and incapacitation times ranged from 2-7 minutes post-injection. When KET was used in elephant seals (500-675 kg), intramuscular doses ranged from 1.4 to 6.9 mg/kg, and incapacitation times ranged from 2 to 25 minutes [35, 36].

This pilot study of KET was subsumed within a larger drug screening study which tested multiple classes of drugs as countermeasures against tetramethylenedisulfotetramine (TETS) poisoning in an oral ingestion model [43]. The subjects described in this report comprise the KET "safety" group of rats, which were not exposed to TETS. These rats received a single KET injection and were then evaluated for 24 hours. Drug doses were chosen based on the published LD_{50} of KET [44], with the intention of testing a wide range of sub-lethal intraperitoneal doses for behavioral and physiological safety. Thus, the data presented here were ancillary to that larger goal and are not intended to compromise a comprehensive study of ketamine safety or its immobilization profile. Nevertheless, the data are of value and provide a strong reference point from which subsequent studies may be initiated.

METHODS

Subjects

Fourteen male Sprague-Dawley rats were obtained from Charles River (SAS SD 400; Wilmington, MA) at 201-225 grams (approximately 7 weeks old) and maintained on a 12 h light/dark cycle. Rats were group housed during acclimation to our facility for five days, then housed individually for the remainder of the study. All experiments were conducted during the light phase. Food restriction was employed to increase the likelihood of rapid consumption of a small piece of food (Froot Loops®; Kellogg Company, Battle Creek, MI) and to ensure an empty stomach during oral TETS exposure. A measured portion of rat chow was provided each afternoon to maintain the subjects at approximately 85% of the free-feeding weights determined from growth curves. The rats weighed between 274.4 to 308.1 g at the time of exposure (*M* = 290.9 g, *SD* = 18.7 g). Water was available *ad libitum* in the rats' home cages. The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense (USAMRICD), and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended. The USAMRICD is a research facility fully accredited by the AAALAC International.

Chemicals and Procedure

Froot Loops (180–220 mg) had 85-95 ul of acetone (>99.5% purchased from Sigma Aldrich) applied approximately 1 hour prior to presentation and consumption, allowing for full evaporation in a certified fume hood. In an observation cage (standard rodent cage measuring 45.7 cm X 24.1 cm X 20.3 cm) with Alpha-Dri bedding, each rat was given a single cereal piece 10 minutes prior to injection of KET. KET (Ketaset©, 100 mg/ml) was obtained from Zoetis (Parsippany, NJ). For the lower doses (37.5 and 75 mg/kg), the KET was diluted with normal saline to 37.5 and 75 mg/ml, respectively. Injection volumes were 1.0 ml/kg for the two lower doses and 1.5 ml/kg for the highest dose (150 mg/kg). All injections were given intraperitoneally using a 1 ml syringe with a 5/8 inch 25 gauge needle.

All experimental animals were randomly assigned, with three rats in each dose group for KET compared to five saline vehicle control animals to properly contextualize food consumption, body weight changes, and shuttlebox avoidance performance. The vehicle control group received saline in a volume of 1 ml/kg, and a total of 5 subjects were summarized for comparisons (weights 285.5 – 329.8 g, *M* = 308.7 g, *SD* =14.3 g).

Shuttlebox Avoidance

Twenty-four hours after agent exposure, animals received training in a two-way active discriminated shuttle box avoidance task. Active avoidance training was conducted in GEMINI test chambers (San Diego Instruments Inc., San Diego, CA) using custom-written Visual Basic 6.0 software (Microsoft Corporation, Redmond, WA). The avoidance session began with a 5-minute acclimation period during which all chamber illumination was off. Fifty discrete trials were presented in each session. At the start of each trial, the photobeam array detected the location of the rat and presented the warning stimulus (WS; a light and 75 (±2) db tone) in that compartment. Failure to ambulate to the darkened compartment within 5 sec resulted in a 1.2 mA scrambled shock applied to the grid floor while the WS continued. Ambulating into the darkened compartment within 15 sec terminated all stimuli (WS, shock) and was registered as an escape response. Failure of the animal to ambulate to the darkened compartment within the 15-second shock duration was recorded as a failed escape or "no response." Shock could be avoided on all trials by promptly ambulating (within 5 sec) into the dark compartment after onset of the WS. Trials were separated by a pseudorandom inter-trial interval (20 ± 5 sec). Immediately following completion of the shuttlebox avoidance test, rats were euthanized with an overdose of a pentobarbital-based solution administered intraperitoneally.

Data Analysis

Statistical analyses were not warranted based upon the small number of subjects and the limited range of doses. Nevertheless, data were orderly enough to discern whether dose-dependent trends existed, and descriptive (non-inferential) statistics (medians, means, and ranges) are reported below.

RESULTS

The main aim of this report was to index measures of immobilization produced by KET. The two primary metrics of immobilization were its latency and duration. The lowest dose (37.5 mg/kg) did not produce immobilization, so neither latency nor duration of immobilization is reported. The left panel of Figure 1 shows the latency between administration of KET and the onset of complete immobilization, defined as sustained absence of movement (except breathing) for one full minute or more. The onset of immobilization was rapid, with all rats succumbing in under four minutes. Considerable overlap in latencies between these two highest KET doses was noted, but there was a trend toward faster immobilization at the highest dose (150 mg/kg), with a median of 2.27 minutes (range of 1.32 to 2.35 minutes) relative to 3.22 minutes (range 2.22 to 3.60 minutes) at the 75 mg/kg dose. These modest differences in immobilization latency stand in stark contrast to differences in immobilization duration, wherein the 75 mg/kg rats exhibited only brief immobilization, approximating only 15.6 minutes (range of 15.57 to 16.28 minutes). The 150 mg/kg rats were immobilized much longer, but with much larger individual differences in response, ranging from 22 to 108 minutes, with a median of 50 minutes. , Thus, a dose-dependent trend was observed in both immobilization latency and duration, but by far the most notable difference was in the duration of immobilization.

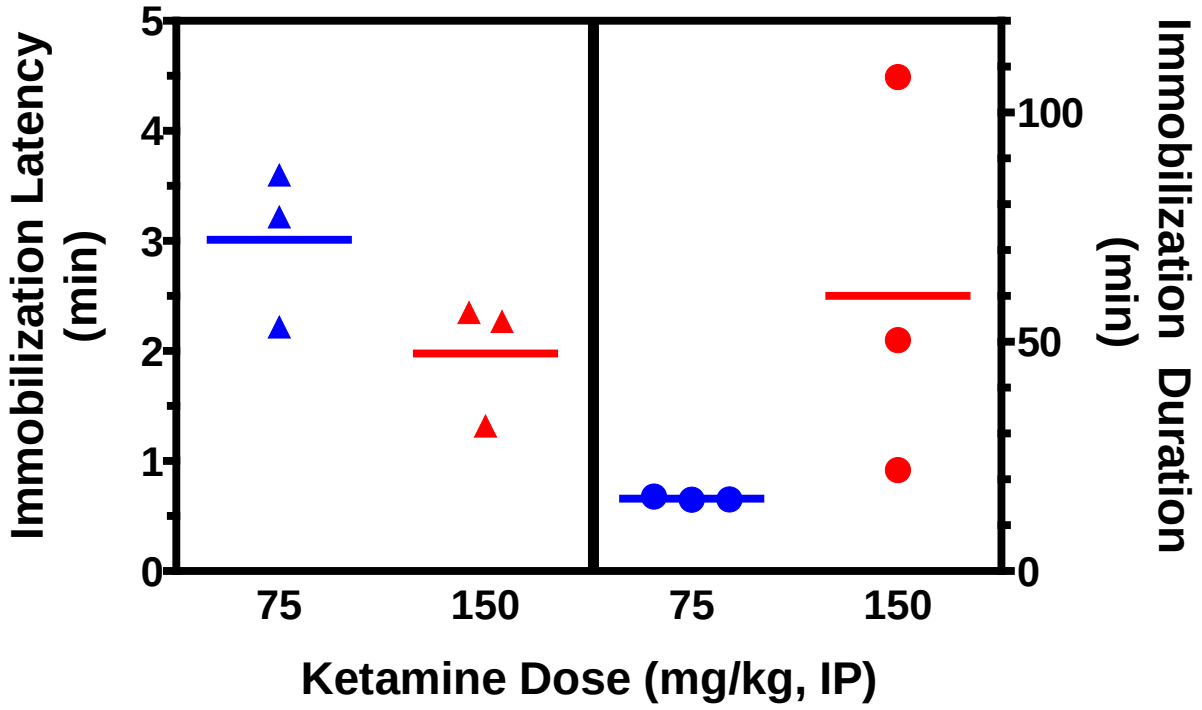

Figure 1. Immobilization latency (left axis; triangles) and duration (right axis; filled circles). Both measures are expressed in minutes. Blue symbols represent individual rats in the 75 mg/kg dose, and red symbols represent the 150 mg/kg dose. Horizontal bars represent the mean.

Figure 2 shows the individual overnight body weight change, expressed as a percentage, for the saline control group and for the three KET dose groups. Only the 150 mg/kg dose produced weight loss in all of the subjects, as the other dose groups showed normal weight gain in the majority of subjects and overlapped considerably with the saline control data. In general, rats ate all food provided to them during the overnight period following the morning of KET exposure, and exhibited normal body weight gain, approximating 1.72% (a 5.19 g increase in body weight). However, the food wastage data can only be discussed to a limited extent because husbandry staff mistakenly discarded any remaining food from two high-dose KET subjects. Unfortunately, these data could have been the most relevant and interesting because all three rats in the 150 mg/kg group exhibited some degree of body weight loss during the overnight period, ranging from 0.51% to 8.06%. Notably, one rat in this high-dose KET group exhibited the median body weight loss of 2.56% (7.2 g) and yet consumed all food provided. Another rat, receiving the 37.5 mg/kg dose, showed a similar pattern, eating all food provided but losing 1.93% (5.3 g). No other rats exhibited body weight loss or food wastage during the overnight period.

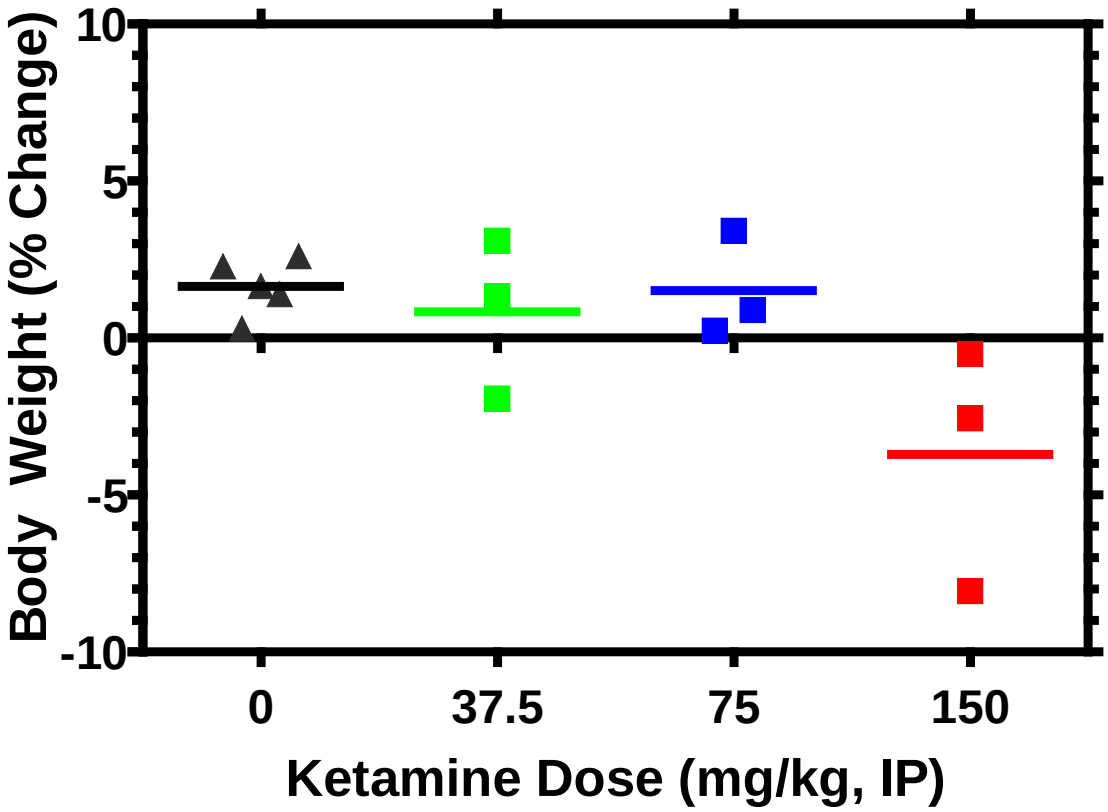

Figure 2. Overnight body weight change, expressed as a percentage of pre-exposure weight, for each KET dose group (green, blue, and red squares) as well as for the saline control group (Dose 0; black triangles). Horizontal bars represent the mean.

Rats in this study were evaluated functionally for acquisition of a discriminated active avoidance response 24 hours after KET or saline injection. All ambulatory activity during the 5-minute acclimation period and throughout the session was recorded. Dependent measures included the percentage of avoidance and escape responses, their respective latencies, and the number of response failures. No such response failures occurred for any animal. The total time spent in the presence of the aversive stimulus was also calculated to summarize any session-level differences in escape latency. There were no observed differences in any of the dependent measures as a function of KET dose. Even at the highest doses, KET-exposed rats appeared to perform comparably to saline controls on the acquisition of the avoidance task, suggesting no lingering motor or sensory effects of KET at 24 hours.

DISCUSSION

The present study characterized the onset and duration of KET-induced immobility and toxicity following IP injection in rats. The 37.5 mg/kg dose of KET did not result in immobilization, and ataxia was the foremost sign displayed by the rats, suggesting a mild state of intoxication that could be of considerable operational relevance. KET produced a rapid onset and dose-dependent duration of immobilization at doses of 75 mg/kg and higher. Onset of immobilization was rapid, occurring within 3-4 minutes or less at the two highest doses. At the 75 mg/kg dose, the duration of immobilization was brief (< 17 minutes), but at the 150 mg/kg dose the duration was much longer and more variable, averaging

60 minutes and ranging from 22 to 108 minutes. Overnight food consumption appeared unaffected, but overnight body weight loss was observed in all three rats at the 150 mg/kg dose. Functional behavioral recovery appeared complete, with no evidence of toxic signs and normal levels of active avoidance acquisition exhibited by all rats at 24 hours after KET or saline injection.

The immobilization data obtained in this small study corroborate and extend past studies of KET in this species. Specifically, the pharmacokinetics of KET in adult Sprague Dawley (SD) rats showed approximately 40% of the peak blood level present at 1 hour following a comparable IP anesthetic dose of KET (125 mg/kg vs. the 150 mg/kg used in our study) [45]. Multiple studies have shown a clear age-dependent effect on KET clearance in SD rats, with older rats showing much slower clearance [45, 46]. It should be noted that intramuscular injections of KET in SD rats may lead to more prolonged intoxication than with IP administrations due to pharmacokinetic differences between the two routes [47]. Also of note is that coadministration of other drugs with KET will sometimes lead to alterations in pharmacokinetics/pharmacodynamics which can have significant effects on the duration and/or depth of KET-induced anesthesia and immobilization [48-50]. This underscores the value of conducting studies (such as this one) wherein KET is the only drug being administered.

The body weight loss observed at the highest KET dose was difficult to interpret due to the husbandry staff error and corresponding loss of data. However, two cases of weight loss occurred even with corroborative data showing that all food was eaten. Such weight loss is hard to explain, but the simplest hypothesis is increased energy expenditure during the overnight period. Whether this increased energy expenditure was due to increased gross activity and wakefulness, or some other factor, will remain unanswered for now. However, KET is well known to produce a potent hypothermia [51, 52], which may result in a compensatory increase in energy expenditure and could be a contributing factor as well.

In general, the present data support the notion that KET is by and large a safe drug even when given at supra-anesthetic doses. Further, the lack of behavioral deficits at 24 hours post-exposure suggests that any lingering drug effects have effectively subsided by that time. Although the results of this study are limited by the relatively small number of animals administered each dose, they provide an important framework for future studies that explore the differences between a wide variety of routes of administration and doses of KET in various animal models. A key recommendation would be the inclusion of more sophisticated behavioral tests soon after KET exposure to more carefully characterize the onset, type, degree, and duration of behavioral impairment and its time-course of recovery. Methods such as those recently reported from our laboratory [53-55] would be ideal for further elaborating KET's important characteristics as a chemical capable of producing prompt and sustained immobilization with minimal long-term effects.

REFERENCES

1. Durieux, M.E., Inhibition by ketamine of muscarinic acetylcholine receptor function. *Anesthesia & Analgesia*, 1995. **81**(1): p. 57-62.
2. Persson, J., Ketamine in pain management. *CNS Neuroscience & Therapeutics*, 2013. **19**(6): p. 396-402.
3. Domino, E.F., Taming the ketamine tiger. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, 2010. **113**(3): p. 678-684.
4. Schneider, P.G. and G. Rodriguez de Lores Arnaiz, Ketamine prevents seizures and reverses changes in muscarinic receptor induced by bicuculline in rats. *Neurochem Int*, 2013. **62**(3): p. 258-64.
5. Martin, L. and D. Smith, Ketamine inhibits serotonin synthesis and metabolism in vivo. *Neuropharmacology*, 1982. **21**(2): p. 119-125.
6. Puu, G., M. Koch, and E. Artursson, Ketamine enantiomers and acetylcholinesterase. *Biochem Pharmacol*, 1991. **41**(12): p. 2043-5.
7. Zanos, P., et al., NMDAR inhibition-independent antidepressant actions of ketamine metabolites. *Nature*, 2016. **533**(7604): p. 481.
8. Kronenberg, R.H., Ketamine as an analgesic: parenteral, oral, rectal, subcutaneous, transdermal and intranasal administration. *Journal of Pain & Palliative Care Pharmacotherapy,* 2002. **16**(3): p. 27-35.
9. Donnelly, R.F., Stability of diluted ketamine packaged in glass vials. *The Canadian Journal of Hospital Pharmacy*, 2013. **66**(3).
10. Chong, C., et al., Development of a sublingual/oral formulation of ketamine for use in neuropathic pain. *Clinical Drug Investigation*, 2009. **29**(5): p. 317-324.
11. Zhu, M.M., et al., Effects of nebulized ketamine on allergen-induced airway hyperresponsiveness and inflammation in actively sensitized Brown-Norway rats. *Journal of Inflammation*, 2007. **4**(1): p. 10.
12. Clements, J. and W. Nimmo, Pharmacokinetics and analgesic effect of ketamine in man. *British Journal of Anaesthesia,* 1981. **53**(1): p. 27-30.
13. White, P., et al., Comparative pharmacology of the ketamine isomers: studies in volunteers. *British Journal of Anaesthesia*, 1985. **57**(2): p. 197-203.
14. White, P.F., et al., Pharmacology of ketamine isomers in surgical patients. *Anesthesiology: The Journal of the American Society of Anesthesiologists*, 1980. **52**(3): p. 231-239.
15. Ihmsen, H., G. Geisslinger, and J. Schüttler, Stereoselective pharmacokinetics of ketamine: R (−)-Ketamine inhibits the elimination of S (+)-ketamine. *Clinical Pharmacology & Therapeutics*, 2001. **70**(5): p. 431-438.
16. Zhang, J.-c., S.-x. Li, and K. Hashimoto, R (−)-ketamine shows greater potency and longer lasting antidepressant effects than S (+)-ketamine. *Pharmacology Biochemistry and Behavior,* 2014. **116**: p. 137-141.
17. Domino, E.F., P. Chodoff, and G. Corssen, Pharmacologic effects of CI-581, a new dissociative anesthetic, in man. *Clinical Pharmacology & Therapeutics,* 1965. **6**(3): p. 279-291.
18. Laskowski, K., et al., A systematic review of intravenous ketamine for postoperative analgesia. *Canadian Journal of Anesthesia*, 2011. **58**(10): p. 911.
19. Monteggia, L.M. and C. Zarate Jr, Antidepressant actions of ketamine: from molecular mechanisms to clinical practice. *Current Opinion in Neurobiology*, 2015. **30**: p. 139-143.
20. Niquet, J., et al., Midazolam-ketamine dual therapy stops cholinergic status epilepticus and reduces Morris water maze deficits. *Epilepsia*, 2016. **57**(9): p. 1406-15.

21. Zeiler, F.A., *Early use of the NMDA receptor antagonist ketamine in refractory and superrefractory status epilepticus. Crit Care Res Pract*, 2015. **2015**: p. 831260.
22. Fang, Y. and X. Wang, Ketamine for the treatment of refractory status epilepticus*. Seizure*, 2015. **30**: p. 14-20.
23. Synowiec, A.S., et al., Ketamine use in the treatment of refractory status epilepticus*. Epilepsy* Res, 2013. **105**(1-2): p. 183-8.
24. Hopper, A.B., et al., Ketamine use for acute agitation in the emergency department*. The Journal of Emergency Medicine*, 2015. **48**(6): p. 712-719.
25. Scheppke, K.A., et al., Prehospital use of im ketamine for sedation of violent and agitated patients*. Western Journal of Emergency Medicine*, 2014. **15**(7): p. 736.
26. CORSSEN, G., et al., Ketamine as the sole anesthetic in open-heart surgery: a preliminary report. *Anesthesia & Analgesia*, 1970. **49**(6): p. 1025-1031.
27. Stefansson, T., I. Wickstrom, and H. Haijamäe, Hemodynamic and metabolic effects of ketamine anesthesia in the geriatric patient*.* A*cta Anaesthesiologica Scandinavica,* 1982. **26**(4): p. 371-377.
28. Rees, D.I. and M.L. Howell, Ketamine-atracurium by continuous infusion as the sole anesthetic for pulmonary surgery*. Anesthesia and Analgesia*, 1986. **65**(8): p. 860-864.
29. Garfield, J.M., M. MA, and F.B. Garfield, A comparison of psychologic responses to ketamine and thiopental-nitrous ovide-halothane*. Anesthesiology*, 1972. **36**(4).
30. Lalonde, B.R. and H.R. Wallage, Postmortem blood ketamine distribution in two fatalities*. Journal of Analytical Toxicology*, 2004. **28**(1): p. 71-74.
31. Peyton, S.H., A.T. Couch, and R.O. Bost, Tissue distribution of ketamine: two case reports*. Journal of Analytical Toxicology*, 1988. **12**(5): p. 268-269.
32. Schifano, F., et al., Trapped in the" K-hole": overview of deaths associated with ketamine misuse in the UK (1993-2006). *Journal of Clinical Psychopharmacology*, 2008. **28**(1): p. 114-116.
33. Gill, J. and M. Stajic, Ketamine in non-hospital and hospital deaths in New York City*. Journal of Forensic Science*, 2000. **45**(3): p. 655-658.
34. Smuts, G., et al., Some practical advantages of CI-581 (ketamine) for the field immobilization of larger wild felines, with comparative notes on baboons and impala*. The Lammgeyer*, 1973. **18**: p. 1-14.
35. Briggs, G.D., R.V. Henrickson, and B.J. Le Boeuf, Ketamine immobilization of northern elephant seals. *Journal of the American Veterinary Medical Association*, 1975. **167**(7): p. 546-8.
36. Mackintosh, C., et al., The immobilization of the badger (Meles meles)*. British Veterinary Journal,* 1976. **132**(6): p. 609-614.
37. Herbst, L.H., C. Packer, and U. Seal, Immobilization of free-ranging African lions (Panthera leo) with a combination of xylazine hydrochloride and ketamine hydrochloride*. Journal of Wildlife Diseases*, 1985. **21**(4): p. 401-404.
38. Hess, L., et al., Experience with a naphthylmedetomidine–ketamine–hyaluronidase combination in inducing immobilization in anthropoid apes*. Journal of Medical Primatology*, 2010. **39**(3): p. 151-159.
39. Fuentes, V., Short term immobilization in the horse with ketamine HCl and promazine HCl combinations*. Equine Veterinary Journal*, 1978. **10**(2): p. 78-81.
40. McLaren, G.W., et al., The use and assessment of ketamine–medetomidine–butorphanol combinations for field anaesthesia in wild European badgers (Meles meles)*. Veterinary Anaesthesia and Analgesia*, 2005. **32**(6): p. 367-372.
41. Bush, M., et al., Use of medetomidine and ketamine for immobilization of free-ranging giraffes. *Journal of the American Veterinary Medical Association*, 2001. **218**(2): p. 245-249.
42. Fernandez-Moran, J., et al., Reversible immobilization of Eurasian otters with a combination of ketamine and medetomidine*. Journal of Wildlife Diseases*, 2001. **37**(3): p. 561-565.

43. Moffett, M.C., et al., Survey of drug therapies against acute oral tetramethylenedisulfotetramine poisoning in a rat voluntary consumption model. *Neurotoxicology*, 2019. **74**: P. 264-271.
44. Budavari, S., et al., *The Merck Index*. Vol. 11. 1989: Merck Rahway, NJ.
45. Veilleux-Lemieux, D., et al., Pharmacokinetics of ketamine and xylazine in young and old Sprague–Dawley rats. *Journal of the American Association for Laboratory Animal Science*, 2013. **52**(5): p. 567-570.
46. Giroux, M.-C., et al., Physiological, pharmacokinetic and liver metabolism comparisons between 3-, 6-, 12-and 18-month-old male Sprague Dawley rats under ketamine-xylazine anesthesia. *Experimental Animals*, 2016. **65**(1): p. 63-75.
47. Veilleux-Lemieux, D., et al., Effects of endotoxemia on the pharmacodynamics and pharmacokinetics of ketamine and xylazine anesthesia in Sprague–Dawley rats. *Veterinary Medicine: Research and Reports*, 2012. **3**: p. 99.
48. Idvall, J., et al., Pharmacodynamic and pharmacokinetic interactions between ketamine and diazepam. *European Journal of Clinical Pharmacology*, 1983. **24**(3): p. 337-343.
49. Edwards, S., C. Minto, and L. Mather, Concurrent ketamine and alfentanil administration: pharmacokinetic considerations. *British Journal of Anaesthesia*, 2002. **88**(1): p. 94-100.
50. Lilius, T., et al., Ketamine coadministration attenuates morphine tolerance and leads to increased brain concentrations of both drugs in the rat. *British Journal of Pharmacology*, 2015. **172**(11): p. 2799-2813.
51. Fahim, I., M. Ismail, and O. Osman, Role of 5-hydroxytryptamine in ketamine-induced hypothermia in the rat. *British Journal of Pharmacology*, 1973. **48**(4): p. 570.
52. Lin, M., C. Chen, and I.-H. Pang, Effect of ketamine on thermoregulation in rats. *Canadian Journal of Physiology and Pharmacology*, 1978. **56**(6): p. 963-967.
53. Rice, N.C., et al., Behavioral intoxication following voluntary oral ingestion of tetramethylenedisulfotetramine: dose-dependent onset, severity, survival, and recovery. *Neurotoxicology*, 2017. **63**: p. 21-32.
54. Rice, N.C., et al., Behavioral toxicity of sodium cyanide following oral ingestion in rats: Dose-dependent onset, severity, survival, and recovery. *Food and Chemical Toxicology*, 2018. **114**: p. 145-154
55. Rice, N.C., et al., Behavioural and physiological assessments of dimethyl trisulfide treatment for acute oral sodium cyanide poisoning. *Basic & Clinical Pharmacology & Toxicology*, 2019. **125**(3): p. 289-303.