The Neurotoxic Potential of a Single Dose of Ketamine in Adolescent and Adult Rats

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Background

In 2019, the FDA approved esketamine (SPRAVATO®), an enantiomer of the dissociative anesthetic ketamine, for treatment-resistant depression and for depressive symptoms associated with acute suicidal ideation or behavior in adults. Ketamine is known to cause age-dependent neurotoxicity in the rat. Data from published literature indicate a single dose of ketamine can result in neuronal necrosis in the adult rat brain and apoptosis in the developing rat brain (equivalent to late gestation up to three years of age in humans). However, data on the use of ketamine during adolescence is lacking. Addressing this knowledge gap will reduce uncertainty about the safe use of ketamine during adolescence. Accordingly, rats were treated with a single dose of ketamine at postnatal day (PND) 21, 30, 35, or 90±3. This age range in rats is roughly equivalent to 4-12 years old, and adulthood, in humans. 72 hours after treatment brains were collected, sectioned, and treated with H&E to investigate the neurotoxic potential of ketamine. In a separate cohort, animals were treated with 100 mg/kg of ketamine to determine the effects of age and sex on the internal dosimetry of ketamine.

Methods

Animals:

Sprague Dawley rats (Charles River) were used for this work. Animals were housed 2-per cage (12-hour light dark cycle) and given ad-libitum access to food and water. Animals in the PND 90 cohort were not time-mated and ranged in age from PND 72-78 at arrival. Animals ranged in age from PND 87 to 93 at time of treatment. Animals in the PND 21-35 group were birthed in-house from purchased animals. Time-mated animals arrived on site as gestational days 9, 10, or 11. Litters were culled to 4 animals per sex on PND 4. Treatments were assigned in a counterbalanced fashion with no more than one treatment assigned per litter per sex. Animals from a litter were only assigned to a single age group. Animals were weaned and tattooed on PND21.

Experimental treatment:

For pathological analysis animals were treated with a single dose of ketamine (50, 75, 100 mg/kg, s.c.; Ketaset Injectable C IIIN, Patterson Veterinary), a positive control (2 or 3 mg/kg MK-801, i.p.; Sigma-Aldrich), or a saline vehicle on postnatal day (PND) 21, 30, 35, or 90. 12 animals were used per treatment group per sex. Time to unresponsiveness and time to normal ambulation were recorded. Any animal still unconscious at the end of the day (4-6pm) was euthanized. All animals were sacrificed after 72 hours with Euthasol, and brains were collected for histopathological evaluation. For the pharmacokinetic studies samples were taken from males at 10, 20, 40, 60, 100, 200, and 300 minutes post injection, and from females at 10, 30, 60, 90, 140, 200, 270, and 360 minutes. Serial sampling was performed via the tail vein of adult animals (n=7), while a single sample was collected via cardiac puncture in PND 21-35 animals (n=7 per time point) as part of the euthanasia process (CO_2).

Tissue processing:

Animals were perfused with heparinized saline followed by 10% neutral buffered formalin. Brains were removed and stored in the fixative for 48 hours. Samples were embedded in paraffin and 5µm thick sections were collected and stained with H&E and evaluated by light microscopy. The brain was sectioned to include the olfactory bulb, fronto-parietal cortex, mid-parietal cortex and thalamus, mid-brain, and cerebellum. The resulting sections were stained with H&E and evaluated for signs of neurodegeneration.

Sample Preparation for Rat Serum:

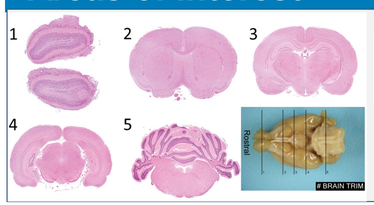
5 µL of rat serum were added to 250 µL of acetonitrile containing the internal standards ketamine-d4 and norketamine-d4. The samples were briefly vortexed and centrifuged at 15,000 rpm for 3 minutes. The supernatant was placed in an HPLC recovery vial for LC/MS/MS analysis.

Analysis of Rat Serum Samples Using LC/MS/MS:

Rat serum samples were analyzed using a Waters Acquity I-class system coupled to a Waters Xevo TQ-S triple quadrupole mass spectrometer (Waters Corporation, Milford, MA) equipped with an electrospray interface operating in positive ion mode. Separation was achieved using a Waters BEH C18 column (2.1 x 50mm), using a 300 µL/min gradient consisting of 10 mM ammonium formate (A) and acetonitrile (B) as follows: 5-95% B in 3.5 min, hold at 95% B for 0.5 min, and return to 5% B in 0.1 min. The column was held at 40 °C, the autosampler was maintained at 18 °C and injection volumes were between 0.2 and 2 µL. The total run time was 6.5 minutes.

Data was acquired in multiple reaction monitoring mode (MRM). The MRM transitions and MS source parameters were optimized for all compounds and transitions and were as follows: 237.93 > 125.06 for ketamine, 242.19 > 128.97 for ketamine-d4, 224.16 > 125.06 for norketamine and 228.16 > 129.02 for norketamine-d4.

Areas of Interest



Areas assessed were based on those proposed by Rao et al., 2011, Tox. Path. However, level 5 here, is the equivalent of level 6 in Rao et al. The figure is adapted from Bolon et al., 2012, Tox. Path.

Ketamine Related Neurotoxicity is Observed in Adult Female Rats

Age	Sex	Region	MK-801 IP 2 mg/kg	MK-801 IP 3 mg/kg	Ketamine SC 100 mg/kg
PND 21	Male	Retrosplenial Cortex		--	--
		Piriform Cortex		--	--
		Entorhinal Cortex		--	--
	Female	Retrosplenial Cortex	--		--
		Piriform Cortex	--		--
		Entorhinal Cortex	--		--
PND 30	Male	Retrosplenial Cortex		1/8 (1.0)	--
		Piriform Cortex		--	--
		Entorhinal Cortex		--	--
	Female	Retrosplenial Cortex	1/3 (1.0)	--	--
		Piriform Cortex	--	--	--
		Entorhinal Cortex	--	--	--
PND 35	Male	Retrosplenial Cortex		1/10 (2.0)	--
		Piriform Cortex		--	--
		Entorhinal Cortex		--	--
	Female	Retrosplenial Cortex	5/5 (1.4)		--
		Piriform Cortex	5/5 (1.0)		--
		Entorhinal Cortex	2/5 (1.0)		--
PND 90	Male	Retrosplenial Cortex		8/8 (1.5)	--
		Piriform Cortex		--	--
		Entorhinal Cortex		--	--
	Female	Retrosplenial Cortex	2/2 (2)		3/12 (1.0)
		Piriform Cortex	1/2 (1.0)		--
		Entorhinal Cortex	2/2 (1.0)		--

The positive control consistently caused neuronal necrosis, however ketamine only caused signs of necrosis in adult female rats. Numbers listed represent how many animals displayed pathology from each group, the number of animals analyzed, and the severity of pathology on a 0-4 scale.

Time to Regain Normal Ambulation (min)

Age	Sex	Ketamine (mg/kg; SC) 50	75	100	MK-801 (mg/kg; IP) 2/3
PND 25	Male	56.50	80.64	129.13	310.22
	Female	68.38	90.89	97.71	318.43
PND 30	Male	54.75	67.63	81.75	264.20
	Female	63.33	97.75	134.83	312.00
PND 35	Male	48.13	65.56	88.38	226.78
	Female	66.60	90.44	146.36	298.29
PND 90	Male	13.90	11.10	8.50	18.55
	Female	11.75	8.70	6.10	14.10

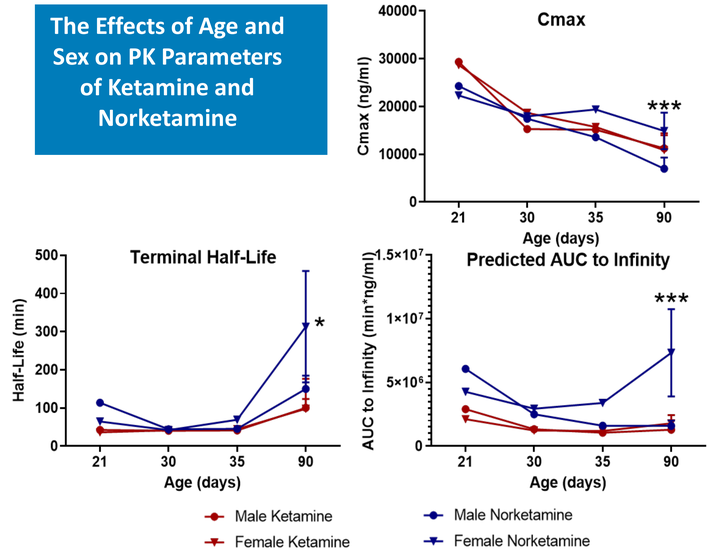
Proportion of Animals Reaching Terminal Sacrifice

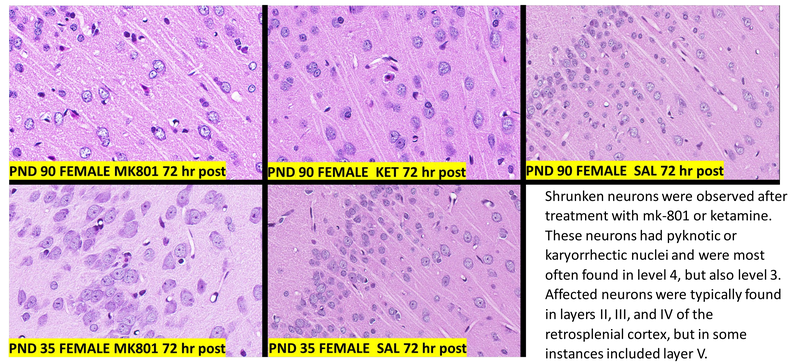
Age	Sex	Veh.	Ketamine (mg/kg; SC) 50	75	100	MK-801 (mg/kg; IP) 3	2
PND 25	Male	1.00	1.00	0.83	0.83	0.75	
	Female	1.00	1.00	1.00	0.75	0.83	0.67
PND 30	Male	1.00	0.92	1.00	0.91	0.73	
	Female	1.00	0.82	1.00	0.82	0.13	1.00
PND 35	Male	1.00	0.92	1.00	0.75	0.83	
	Female	1.00	1.00	1.00	0.92	0.00	0.71
PND 90	Male	1.00	0.92	0.92	1.00	0.67	
	Female	1.00	0.92	1.00	1.00	0.00	0.33

Results

Necrotic neurons were observed after treatment with the positive control (MK-801) in both male and female animals at PND 30, 35, and 90. However, ketamine (100 mg/kg, s.c.) only caused necrosis in the retrosplenial cortex of female rats dosed on PND 90 (N=3/12). Age-dependent effects were observed in the pharmacokinetics of ketamine. Younger animals had higher estimated Cmax values than those observed in the PND animals. Surprisingly, the norketamine AUC was much higher in PND90 females when compared to males. This effect was driven by the longer terminal half-life of norketamine in adult female rats. Younger animals also had higher peak levels of ketamine when compared to the PND 90 group.

The Effects of Age and Sex on PK Parameters of Ketamine and Norketamine





Shrunken neurons were observed after treatment with mk-801 or ketamine. These neurons had pyknotic or karyorrhectic nuclei and were most often found in level 4, but also level 3. Affected neurons were typically found in layers II, III, and IV of the retrosplenial cortex, but in some instances included layer V.

Conclusions

Adolescent rats had higher observed blood levels of ketamine. Despite this, no signs of ketamine-induced neurotoxicity were observed in the adolescent groups. Neuronal necrosis was only observed in adult female rats. These data show that younger Sprague Dawley rats are less vulnerable to the neurotoxic effects of ketamine on both a mg/kg basis and in terms of blood plasma levels. It is unclear why necrosis was only observed in female rats. We speculate this is related to the increased half-life of elimination / AUC of norketamine in female rats. This could explain why necrosis was not observed in adolescent rats despite having higher serum levels of ketamine when compared to adults.

These data support the idea that there is a "protected period" in young rats to the toxic effects of ketamine. However, it is unclear if this is driven by a reduced sensitivity to ketamine-induced cell death, or lower levels of norketamine. Regardless, these data suggest that adolescent rats are not more sensitive to the neurotoxic effects of ketamine. Moreover, norketamine levels should be taken into consideration in future studies of ketamine-related neurotoxicity.

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