

# Effects of endotoxemia on the pharmacodynamics and pharmacokinetics of ketamine and xylazine anesthesia in Sprague–Dawley rats

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**Purpose:** To evaluate the effects of endotoxemia on the pharmacokinetics and pharmacodynamics of ketamine and xylazine anesthesia in Sprague–Dawley rats.

**Methods:** Sprague–Dawley rats received ketamine (80 mg/kg) and xylazine (5 mg/kg) intramuscularly following the intraperitoneal administration of different lipopolysaccharide concentrations (1, 10, and 100 µg/kg) to simulate different levels of endotoxemia. Results were compared to control animals receiving saline intraperitoneally. During anesthesia, a toe pinch was performed to evaluate anesthesia duration, and selected physiological parameters (heart and respiratory rates, oxygen saturation, and rectal temperature) were taken. Blood samples were also taken during anesthesia at selected time points for the analysis of plasmatic ketamine and xylazine concentrations by liquid chromatography–mass spectrometry. Blood samples were taken 1 week prior to and 24 hours following anesthesia for blood biochemistry.

**Results:** Anesthesia duration significantly increased for moderate (10 µg/kg) and high (100 µg/kg) lipopolysaccharide groups. Liver histopathology showed minor to moderate necrosis in all lipopolysaccharide groups in some animals. The most important physiological change that occurred was a decrease in oxygen saturation, and for blood biochemistry a decrease in serum albumin. Ketamine pharmacokinetics were not affected except for the moderate (10 µg/kg) lipopolysaccharide group where a decrease in the area under the plasma concentration–time curve from time zero to the last measurable concentration, a decrease in half-life, and an increase in the clearance were observed. For xylazine, the area under the plasma concentration–time curve increased and the clearance decreased in the moderate (10 µg/kg) and high (100 µg/kg) lipopolysaccharide groups.

**Conclusion:** During ketamine–xylazine anesthesia, endotoxemia may alter xylazine pharmacokinetics and selected biochemical and physiological parameters, suggesting that anesthetic drug dosages could be modified for a more rapid recovery.

**Keywords:** lipopolysaccharide, ketamine, xylazine, pharmacokinetics, rats

## Introduction

Fever is a common clinical sign caused by endogenous or exogenous pyrogens and it is often associated with bacterial and viral infections. Lipopolysaccharides (LPS) are constituents of the bacterial cell wall that induces fever in animals.<sup>1</sup> This exogenous pyrogen, which is a component of Gram-negative bacteria membranes, indirectly stimulates the production of inflammatory cytokines such as tumor necrosis factor- $\alpha$ , interleukins, and interferons, which are endogenous pyrogens.<sup>2,3</sup> These cytokines induce important functional modifications that affect different organs including the liver and the kidney.<sup>4,5</sup> Pyrogens may decrease the metabolism of drugs by their action on liver

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cytochrome P450 in all animal species including rats.<sup>6,7</sup> Renal excretion can also be altered since glomerular filtration rate and plasmatic renal flow are both reduced following pyrogen administration.<sup>8</sup> Since endotoxemia may cause a decrease in liver metabolism and renal filtration, plasmatic concentrations of drugs could be altered with diseases or infections.<sup>3</sup>

Both xylazine – an  $\alpha_2$ -agonist that reduces noradrenergic activity in the brain and has sedative and antinociceptive properties – and ketamine – an N-methyl-D-aspartate antagonist that has a generalized depressant effect in all parts of the central nervous system, which explains its anesthetic properties – are used in combination to anesthetize rodents.<sup>9</sup> The combination of xylazine and ketamine is often considered as a first choice of injectable anesthetics.<sup>10–12</sup> They are rapidly absorbed after an intramuscular injection and well distributed to the central nervous system.<sup>13</sup> Both are metabolized by the liver and excreted mainly by the kidney.<sup>10</sup> However, little is known about the effects of endotoxins on the metabolism and excretion of these drugs.

The main objective of this study was to evaluate the pharmacokinetics and physiological changes associated with the administration of ketamine and xylazine in rats when administered at an anesthetic dose following the administration of different LPS concentrations. Previous experiments have used various LPS concentrations to induce fever.<sup>14–16</sup> Hansen et al showed that an intraperitoneal injection of LPS (1, 10, and 50  $\mu\text{g}/\text{kg}$ ) induced fever in adult male Sprague–Dawley rats.<sup>1</sup> Low (1  $\mu\text{g}/\text{kg}$ ), moderate (10  $\mu\text{g}/\text{kg}$ ), and high (100  $\mu\text{g}/\text{kg}$ ) doses of LPS were used to observe if there was a dose–effect relationship between endotoxemia and the pharmacological and physiological responses of ketamine–xylazine anesthesia.

## Materials and methods

### Animals and husbandry

Twenty-four male Sprague–Dawley rats (Charles River Laboratories, Saint-Constant, Canada) weighing between 300–350 g (approximately 8–9 weeks of age) were used for this study. After their arrival, animals were allowed 7 days of acclimation before the start of the experiments. They were housed in a standard laboratory animal environment (fresh filtered air, 15 changes/hour; temperature, 22°C–24°C; humidity, 40%–60%; and light–dark cycle, 12:12 hours). The rats were group-housed (two rats/cage) in polycarbonate cages (Ancare, Bellmore, NY) on hardwood bedding (7090C Teklad Certified Sani-Chips; Harlan Laboratories, Madison, WI). They received tap water and a certified laboratory diet (2018 Teklad Global Rodent Diet<sup>®</sup>; Harlan Laboratories)

ad libitum. The experimental protocol was approved by the Laval University Institutional Animal Care and Use Committee (Quebec, Canada) prior to animal use, in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, Canada).

### Treatments

The control group (n = 6 rats) received 0.1 mL of pyrogen-free saline intraperitoneally (0.9% sodium chloride; Hospira, Inc, Lake Forest, IL) and each experimental group (n = 6 rats/LPS concentration) received a 0.1 mL intraperitoneal injection of either 1, 10, or 100  $\mu\text{g}/\text{kg}$  of LPS to simulate three levels of endotoxemia.<sup>1</sup> LPS (*Escherichia coli* 011:B4; Sigma-Aldrich, St Louis, MO) and sterile saline were injected 2 hours prior to the ketamine–xylazine injection. LPS was dissolved in sterile pyrogen-free saline (Hospira) at an ambient temperature (22°C–24°C). Ketamine (80 mg/kg; Ketalean; Bimeda-MTC, Cambridge, Canada) and xylazine (5 mg/kg; Xylamax; Bimeda-MTC) were administered intramuscularly in the hip extensor (semitendinosus and semimembranosus muscles).

### Evaluation of physiological parameters and the withdrawal reflex

Baseline physiological measures and paw withdrawal response were taken 30 minutes before LPS administration. Prior to each blood collection (approximately 1 minute), body temperature, cardiac and respiratory frequencies, and blood oxygen saturation data were collected. Rectal temperature was monitored (TH-8 Thermalert; Physitemp Instruments, Inc, Clifton, NJ) and a small animal oximeter (8500 Handheld Pulse Oximeter; Nonin Medical, Inc, Plymouth, MN) was used for cardiac frequency and blood oxygen saturation monitoring. Respiratory frequency was monitored by direct observation and the withdrawal reflex was monitored by the reaction to a toe pinch. The toe pinch was performed by pressing the interdigital hind paw skin with hemostatic forceps prior to anesthesia and starting at 15 minutes after ketamine–xylazine administration continuing at each blood collection time point until a positive reaction (hind leg movement) was noted.

### Blood biochemistry and histological preparations

Under isoflurane anesthesia, blood (1 mL) was collected from the jugular vein of each rat 1 week prior to and 24 hours after the ketamine–xylazine injection. It was placed in 1 mL serum tubes (Becton Dickinson, and Company, Franklin Lakes, NJ)

for the evaluation of selected biochemical parameters with a Synchron CX<sup>®</sup>5 Pro Clinical System (Beckman Coulter, Brea, CA). Parameters included glucose, blood urea nitrogen, creatinine, alanine aminotransferase, alkaline phosphatase, total protein, albumin, and globulins. Experimental values were compared with normal ranges taken from published findings.<sup>17</sup>

Following euthanasia, the kidney and liver were collected and preserved in a buffered 10% formalin solution prior to histological preparations (staining with hematoxylin–eosin–safron). Specimens were sent to the pathology department of the Faculty of Veterinary Medicine of the University of Montreal (Saint-Hyacinthe, Canada).

### Blood sampling for the pharmacokinetic study and urine collections

Following ketamine–xylazine administrations, the animals were housed in rat metabolic cages (Tecniplast SpA, Buguggiate, Italy) for urine collection over 24 hours to evaluate total urinary volume and renal excretion of ketamine and xylazine. The excretion of both drugs is reported as the excreted fraction (ie, the total drug excreted – total drug administered). The total drug excreted was calculated from the urine concentration measured by high performance liquid chromatography (HPLC; Series 200; PerkinElmer, Waltham, MA), tandem mass spectrometer (Applied Biosystems/MDS Sciex PE Sciex API III+; Life Technologies, Carlsbad, CA) and total urinary output over 24 hours.

Prior to each jugular vein blood collection, animals were placed on a circulating hot water blanket. Blood samples (0.3 mL/time points) for the pharmacokinetic study were collected under isoflurane anesthesia (0.5 L/minute oxygen), with the use of a mask, at 5, 15, and 30 minutes and 1, 2, 6, and 24 hours after ketamine–xylazine administration. The total time for each blood collection was approximately 1 minute. Blood was collected in 1 mL BD Microtainer<sup>®</sup> tubes with potassium ethylenediaminetetraacetic acid (Becton Dickinson). Samples were maintained on ice and centrifuged (3200 g for 10 minutes) within 30 minutes of collection. Immediately following the last blood collection, the rats were euthanized by isoflurane overdose. All samples were kept at –80°C pending analysis by HPLC tandem mass spectrometer.

### Bioanalytical method

The analysis of ketamine and xylazine was performed using an HPLC tandem mass spectrometer. Ketamine and xylazine were extracted from rat plasma using a protein

precipitation method. Each plasma sample (50 µL) was mixed with 250 µL of internal standard solution (100 ng/mL of dextromethorphan in an acetone/methanol ratio of 50:50) in a 1.5 mL centrifuge tube. For the urine samples, 50 µL of urine was mixed with 1 mL of the internal standard solution. Samples were vortexed, allowed to rest 10 minutes, and then centrifuged (12,000 g for 10 minutes). Using an isocratic mobile phase with a Hypersil Phenyl 100 × 2 mm column (Thermo Fisher Scientific, Waltham, MA), 200 µL of the supernatant was used for chromatographic separation. The mobile phase consisted of acetonitrile, methanol, and 0.5% formic acid in water at a ratio of 60:20:20, respectively, with a fixed flow rate of 0.3 mL/minute; 2 µL of the extracted sample was injected and the total run time was set to 3 minutes. The mass spectrometer was interfaced with the HPLC system using a pneumatic assisted electrospray ion source. The nitrogen pressure of the nebulizer gas was set at 40 psi and the electrospray ionization electrode was set to 4000 V. The declustering potential was set at 15 V and the laboratory collision energy at 22.5 V. The collision gas used was argon. The selected reaction monitoring transitions were  $m/z$  238→163, 221→90, and 272→215 for ketamine, xylazine, and dextromethorphan, respectively. The analytical ranges used were 0.1–25 µg/mL for ketamine and 0.01–5.0 µg/mL for xylazine. Observed coefficients of determination were  $\geq 0.9987$  and  $\geq 0.9976$  for ketamine and xylazine, respectively. The precisions obtained ranged from 2.3%–9.2% and 1.5%–6.9% for ketamine and xylazine, respectively. Likewise, the accuracy observed was 96.9%–100.5% and 92.2%–111.7% for ketamine and xylazine, respectively. The lower limit of quantitation was set at 0.1 µg/mL and 0.01 µg/mL for ketamine and xylazine, respectively.

### Pharmacokinetics

Pharmacokinetic parameters of ketamine and xylazine in plasma were calculated using noncompartmental methods.<sup>18</sup> The area under the curve from time zero to the last measurable concentration ( $AUC_{0-t}$ ) was calculated using the linear trapezoidal rule. A terminal rate constant of elimination was calculated using a minimum of three measurable plasma concentrations and a terminal elimination half-life was calculated using  $0.693/k_{el}$ . All parameters were confirmed by using a one-compartment model using Pharsight<sup>®</sup> WinNonlin<sup>®</sup> version 5.3 (Certara, St Louis, MO). The correlation between fitted and observed concentrations for ketamine and xylazine was 0.9840 and 0.9869, respectively. Reported pharmacokinetic values are from WinNonlin.

## Statistical analyses

Duration of the ketamine–xylazine anesthesia was evaluated using a chi-squared statistical test with a priori contrasts to see if there were differences when comparing LPS groups to controls. All hematologic and biochemistry data were analyzed statistically by using *t*-tests. Pre–post anesthesia values were compared for all groups. Parametric repeated measures analysis of variance and Tukey's post hoc analysis were used for physiologic and pharmacologic data. Statistical significance was set at  $P < 0.05$ .

## Results

Prior to the ketamine–xylazine administration, rats receiving 1  $\mu\text{g}/\text{kg}$  of LPS appeared normal. Rats receiving 10  $\mu\text{g}/\text{kg}$  were slightly amorphous, showing decreased exploratory movement in the cage compared to the controls and the 1  $\mu\text{g}/\text{kg}$  LPS group; however, a slightly hunched posture with piloerection and a clear decrease of spontaneous movement was apparent with 100  $\mu\text{g}/\text{kg}$ .

## Anesthesia duration

Anesthesia duration, demonstrated by the percentage of Sprague–Dawley rats showing a positive hind paw pinch reaction, following ketamine–xylazine administration is presented in Figure 1. The toe pinch reflex was absent in these animals at 5 minutes and 15 minutes; however, at the

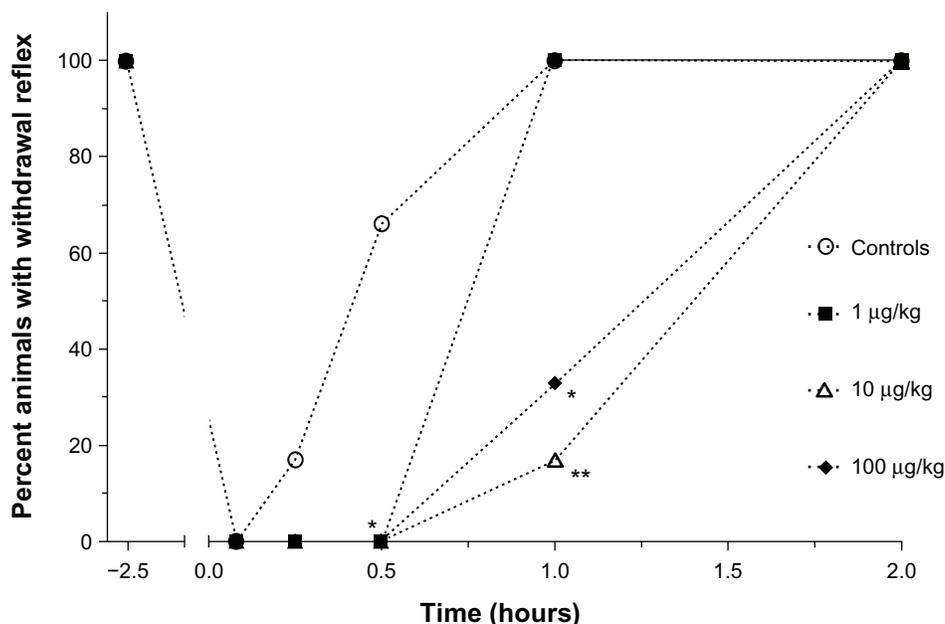
30-minute time point, animals receiving 1  $\mu\text{g}/\text{kg}$  LPS were still anesthetized ( $P < 0.05$ ) when compared to controls. In these animals, the withdrawal reflex was again present 1 hour following ketamine–xylazine administration. All animals that received 10  $\mu\text{g}/\text{kg}$  or 100  $\mu\text{g}/\text{kg}$  of LPS had a toe pinch reflex 2 hours after ketamine–xylazine injection, but these groups were statistically different from the controls at 1 hour ( $P < 0.01$  and  $P < 0.05$ , respectively), as most animals were still anesthetized at this time point. Therefore, the control animals and animals receiving the low LPS concentration had a shorter anesthesia duration than animals receiving the moderate and high LPS concentrations.

## Physiological changes

Rectal temperature was significantly lower with 1  $\mu\text{g}/\text{kg}$  LPS at 2 hours and 6 hours ( $P < 0.0001$ ) and at 2 hours with 10  $\mu\text{g}/\text{kg}$  LPS ( $P < 0.05$ ) in comparison to the controls (Figure 2). No significant difference was found with the 100  $\mu\text{g}/\text{kg}$  LPS administration.

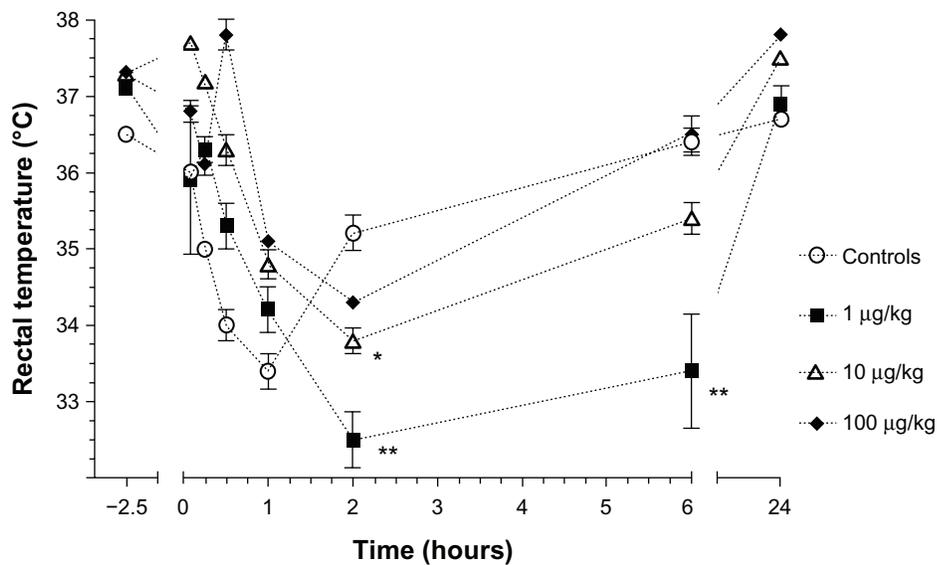
No changes in respiratory frequency occurred with different LPS administrations except at 6 hours when all LPS groups showed a lower respiratory frequency in comparison to the controls ( $P < 0.05$ ) (Figure 3).

The effects of LPS during anesthesia on cardiac frequency are presented in Figure 4. The high LPS group had a higher



**Figure 1** Percentage of Sprague–Dawley rats ( $n = 6/\text{group}$ ) showing a positive hind paw pinch reaction 30 minutes prior to lipopolysaccharide administrations (2.5 hours prior to the administration of anesthetics) and starting at 15 minutes after administration of ketamine and xylazine continuing at each blood collection time point (5, 15, and 30 minutes and 1, 2, 6, and 24 hours).

**Note:** Animals received either saline (controls) or different concentrations of *Escherichia coli* lipopolysaccharide (1, 10, or 100  $\mu\text{g}/\text{kg}$ ) injected intraperitoneally 2 hours before a ketamine (80  $\text{mg}/\text{kg}$ ) and xylazine (5  $\text{mg}/\text{kg}$ ) intramuscular injection.



**Figure 2** Mean ( $\pm$ standard error) rectal temperature in Sprague–Dawley rats ( $n = 6$ /group) prior to polysaccharide administrations (2.5 hours prior to the administration of anesthetics) and following an intramuscular injection of ketamine (80 mg/kg) and xylazine (5 mg/kg) at 5, 15, and 30 minutes and 1, 2, 6, and 24 hours in animals that received either saline (controls) or different concentrations of *Escherichia coli* lipopolysaccharide (1, 10, or 100  $\mu$ g/kg) injected intraperitoneally 2 hours before anesthesia. **Notes:** \* $P < 0.05$ ; \*\* $P < 0.0001$ .

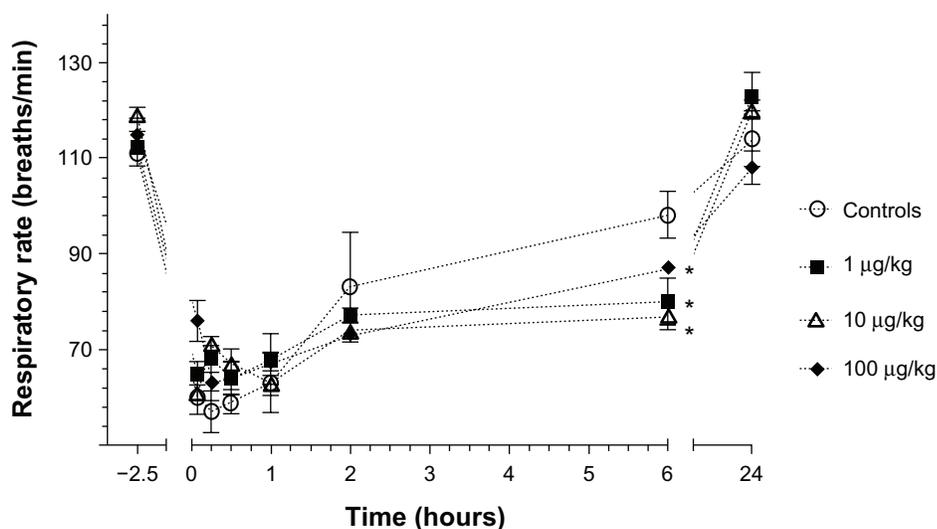
cardiac frequency at 1 hour and 2 hours ( $P < 0.0001$ ), and at the 6-hour time point the 1  $\mu$ g/kg ( $P < 0.0001$ ) and the 10  $\mu$ g/kg ( $P < 0.01$ ) group had a lower cardiac frequency in comparison to the controls. At 24 hours following a 1  $\mu$ g/kg LPS injection, the cardiac frequency was still lower than the controls ( $P < 0.01$ ).

Blood oxygen saturation was significantly lower during the first 30 minutes for the low and moderate LPS groups ( $P < 0.0001$ ), and less so at 1 hour ( $P < 0.05$ ). The high

LPS group had an increased oxygen saturation at 5 minutes ( $P < 0.05$ ) and decreased oxygen saturation at 15 minutes and 30 minutes ( $P < 0.05$ ) when compared to the controls (Figure 5).

### Biochemical and pathological changes

Biochemical results are presented in Table 1. All pre–post anesthesia values are within normal limits except for plas-matic albumin. The decrease of albumin with anesthesia



**Figure 3** Mean ( $\pm$ standard error) respiratory frequency in Sprague–Dawley rats ( $n = 6$ /group) prior to lipopolysaccharide administrations (2.5 hours prior to the administration of anesthetics) and following an intramuscular injection of ketamine (80 mg/kg) and xylazine (5 mg/kg) at 5, 15, and 30 minutes and 1, 2, 6, and 24 hours in animals that received either saline (controls) or different concentrations of *Escherichia coli* lipopolysaccharide (1, 10, or 100  $\mu$ g/kg) injected intraperitoneally 2 hours before anesthesia. **Note:** \* $P < 0.05$ .

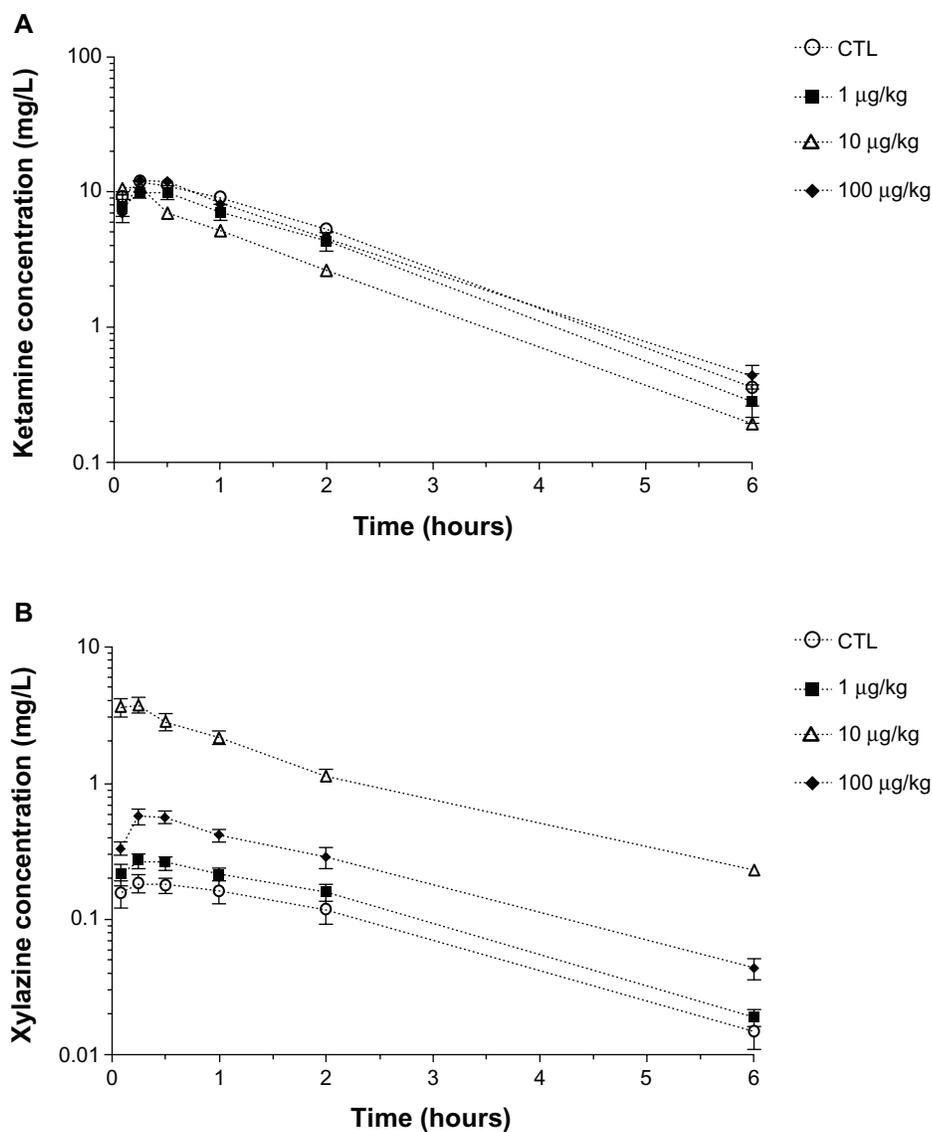


**Table 1** Mean ( $\pm$ standard deviation) biochemical parameters in Sprague–Dawley rats ( $n = 6$ /group) from a blood collection (1 mL) taken 1 week prior to and 24 hours after an intramuscular injection of ketamine (80 mg/kg) and xylazine (5 mg/kg) in controls (saline intraperitoneally) and in animals that received *Escherichia coli* lipopolysaccharide (1, 10, or 100  $\mu$ g/kg intraperitoneally) injected 2 hours before anesthesia

Parameters	Normal values	Control group		LPS 1 $\mu$ g/kg		LPS 10 $\mu$ g/kg		LPS 100 $\mu$ g/kg	
		Before	After	Before	After	Before	After	Before	After
Glucose (mmol/L)	4.4–16.7	10.2 (1.58)	4.3 (3.22)	8.6 (0.50)	8.8 (1.13)	10.2 (1.04)	8.8 (1.23)	8.2 (0.65)	9.1 (1.78)
BUN (mmol/L)	3.9–8.2	5.39 (0.47)	5 (0.77)	5.19 (0.54)	4.20 (1.56)	5.06 (0.32)	4.04 (0.48)	4.8 (0.32)	8.28 (6.24)
Creatinine ( $\mu$ mol/L)	35–123	33 (4.3)	37 (6.30)	31 (2.10)	33 (4.49)	31 (1.75)	34.5** (3.02)	28 (1.03)	34* (5.18)
ALT (U/L)	28–40	38 (6.30)	58 (12.24)	53 (5.64)	57 (8.80)	52 (4.17)	57 (9.05)	56 (10.43)	148 (105.4)
Total protein (g/L)	58–66	53.5 (4.19)	51.3 (2.74)	55.0 (2.72)	48.3** (3.75)	53.4 (3.04)	51.8 (2.41)	53.2 (1.46)	47.3** (1.95)
Albumin (g/L)	33–46	31.6 (1.13)	28.3** (1.77)	33.0 (1.22)	27.7** (1.58)	31.8 (0.98)	28.0** (1.15)	32.6 (0.78)	25.9** (0.89)
Globulins (g/L)	17–30	21.9 (3.22)	23 (1.93)	22.0 (1.60)	20.6 (2.33)	21.7 (2.24)	23.8 (1.60)	20.6 (0.82)	21.5 (1.35)

Notes: \* $P \leq 0.05$ ; \*\* $P \leq 0.01$ .

Abbreviations: ALT, alanine aminotransferase; BUN, blood urea nitrogen; LPS, lipopolysaccharide.



**Figure 6** Mean ( $\pm$ standard error) concentration–time profiles of (A) ketamine and (B) xylazine in plasma following an intramuscular administration of ketamine (80 mg/kg) and xylazine (5 mg/kg) in combination with an intraperitoneal administration of either saline (controls;  $n = 6$  rats) or different concentrations of *Escherichia coli* lipopolysaccharide (1, 10, or 100  $\mu$ g/kg;  $n = 6$  rats/lipopolysaccharide concentration) 2 hours before anesthesia.

Abbreviation: CTL, controls.

**Table 2** Mean ( $\pm$ standard error) pharmacokinetic parameters of ketamine for controls and lipopolysaccharide groups (Sprague–Dawley rats,  $n = 6$ /group) that received either saline (controls) or *Escherichia coli* lipopolysaccharide (1, 10, or 100  $\mu\text{g}/\text{kg}$ ) injected intraperitoneally 2 hours before an intramuscular injection of ketamine (80  $\text{mg}/\text{kg}$ ) and xylazine (5  $\text{mg}/\text{kg}$ )

Parameters	Control	1 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$	100 $\mu\text{g}/\text{kg}$
$\text{AUC}_{0-t}$ (mg-hour/L)	25.82 $\pm$ 1.88	20.87 $\pm$ 3.08	15.35 $\pm$ 0.77*	23.99 $\pm$ 1.93
CL/F (L/hour/kg)	3.18 $\pm$ 0.24	4.22 $\pm$ 0.52	5.28 $\pm$ 0.26*	3.44 $\pm$ 0.28
$T_{1/2}$ (hours)	1.20 $\pm$ 0.05	1.09 $\pm$ 0.09	0.88 $\pm$ 0.02*	1.09 $\pm$ 0.08
$T_{\text{max}}$ (hours)	0.27 $\pm$ 0.03	0.30 $\pm$ 0.03	0.14 $\pm$ 0.02*	0.34 $\pm$ 0.04
$C_{\text{max}}$ (mg/L)	12.65 $\pm$ 0.77	10.58 $\pm$ 1.16	10.83 $\pm$ 0.66	12.40 $\pm$ 1.05

Note: \* $P \leq 0.0001$ .

**Abbreviations:**  $\text{AUC}_{0-t}$ , area under the plasma concentration–time curve from time zero to the last measurable concentration; CL/F, total clearance of the drug after oral administration;  $C_{\text{max}}$ , maximum plasma drug concentration;  $T_{1/2}$ , half-life;  $T_{\text{max}}$ , time taken to reach the maximum plasma drug concentration.

Mean pharmacokinetic parameters for xylazine are presented in Table 3. Mean pharmacokinetic parameters of xylazine of the 1  $\mu\text{g}/\text{kg}$  LPS group were not significantly different from the control group. An increase in  $\text{AUC}_{0-t}$  and maximum plasma concentration were seen for the 10  $\mu\text{g}/\text{kg}$  and 100  $\mu\text{g}/\text{kg}$  LPS groups ( $P < 0.0001$ ). A decrease in xylazine clearance was also observed in both groups ( $P < 0.0001$ ). These changes were more important for the group that received 10  $\mu\text{g}/\text{kg}$  LPS in comparison to 100  $\mu\text{g}/\text{kg}$  LPS.

Urinary volumes collected over 24 hours for the 1  $\mu\text{g}/\text{kg}$  and 10  $\mu\text{g}/\text{kg}$  LPS groups (mean = 16  $\pm$  7 mL) did not differ significantly from the controls (mean = 12  $\pm$  3 mL). However, the urinary output significantly increased following the administration of 100  $\mu\text{g}/\text{kg}$  LPS (mean = 33  $\pm$  15 mL;  $P < 0.01$ ). The excreted fractions of ketamine and xylazine with different LPS administration are presented in Table 4. For ketamine, only the administration of 100  $\mu\text{g}/\text{kg}$  LPS caused a significant increase in the excreted fraction ( $P < 0.01$ ). For xylazine, both the 10  $\mu\text{g}/\text{kg}$  ( $P < 0.001$ ) and 100  $\mu\text{g}/\text{kg}$  ( $P < 0.05$ ) LPS administration caused an increase in the excreted urinary fraction.

## Discussion

Increasing concentrations of LPS during ketamine–xylazine anesthesia in Sprague–Dawley rats caused significant

pharmacokinetic, biochemical, and pathophysiological changes. Anesthesia duration increased; however, this cannot be explained by physiological changes. Increased duration could be related to other factors such as drug metabolism or the drug tissue availability, which may change with endotoxemia. To generalize the current findings, these studies should be done in rats of different gender, species, and age before they can be broadly applied to different experimental models.

In intact animals, LPS causes a release of endogenous pyrogens that produces fever,<sup>19</sup> which increases metabolism and oxygen demand. A mainly hypothermic response was seen with LPS administration. Thermoregulatory responses to infection are well studied in the rat, and in both rats and humans, infection can induce febrile, hypothermic, or mixed hypothermic and febrile responses.<sup>20</sup> The cardiovascular system responds by increasing heart rate and peripheral vasodilatation to increase skin blood flow, allowing heat dissipation.<sup>21</sup> In the current experiment, LPS had little effect on early postanesthesia cardiac frequency except for the 100  $\mu\text{g}/\text{kg}$  LPS dose, which caused an increase in heart rate. In many species, the ketamine–xylazine combination is known to decrease the heart rate.<sup>22–24</sup> The current results suggest that this effect could only be overcome with high LPS concentration.

**Table 3** Mean ( $\pm$ standard error) pharmacokinetic parameters of xylazine for controls and lipopolysaccharide groups (Sprague–Dawley rats,  $n = 6$ /group) that received either saline (controls) or *Escherichia coli* lipopolysaccharide (1, 10, or 100  $\mu\text{g}/\text{kg}$ ) injected intraperitoneally 2 hours before an intramuscular injection of ketamine (80  $\text{mg}/\text{kg}$ ) and xylazine (5  $\text{mg}/\text{kg}$ )

Parameters	Saline	1 $\mu\text{g}/\text{kg}$	10 $\mu\text{g}/\text{kg}$	100 $\mu\text{g}/\text{kg}$
$\text{AUC}_{0-t}$ (mg-hour/L)	0.54 $\pm$ 0.11	0.74 $\pm$ 0.09	7.11 $\pm$ 0.68*	1.44 $\pm$ 0.2*
CL/F (L/hour/kg)	10.99 $\pm$ 2.38	7.35 $\pm$ 1.13	0.74 $\pm$ 0.09*	3.85 $\pm$ 0.55*
$T_{1/2}$ (hours)	1.59 $\pm$ 0.13	1.62 $\pm$ 0.06	1.34 $\pm$ 0.11	1.38 $\pm$ 0.07
$T_{\text{max}}$ (hours)	0.27 $\pm$ 0.03	0.28 $\pm$ 0.03	0.19 $\pm$ 0.02	0.34 $\pm$ 0.04
$C_{\text{max}}$ (mg/L)	0.20 $\pm$ 0.03	0.28 $\pm$ 0.04	3.69 $\pm$ 0.52*	0.58 $\pm$ 0.06*

Note: \* $P \leq 0.0001$ .

**Abbreviations:**  $\text{AUC}_{0-t}$ , area under the plasma concentration–time curve from time zero to the last measurable concentration; CL/F, total clearance of the drug after oral administration;  $C_{\text{max}}$ , maximum plasma drug concentration;  $T_{1/2}$ , half-life;  $T_{\text{max}}$ , time taken to reach the maximum plasma drug concentration.

**Table 4** Mean ( $\pm$ standard error) fractional excretion of ketamine and xylazine following a 24-hour urine collection for control animals (saline intraperitoneally) and animals that received *Escherichia coli* lipopolysaccharide (1, 10, or 100  $\mu$ g/kg intraperitoneally) 2 hours before an intramuscular injection of ketamine (80 mg/kg) and xylazine (5 mg/kg)

Groups	FE ketamine	FE xylazine
Saline	1.4 $\pm$ 0.4	0.6 $\pm$ 0.1
1 $\mu$ g/kg	1.9 $\pm$ 0.2	1.0 $\pm$ 0.2
10 $\mu$ g/kg	2.4 $\pm$ 0.3	16.1 $\pm$ 2.9
100 $\mu$ g/kg	3.8 $\pm$ 0.5	5.1 $\pm$ 2.0

**Abbreviation:** FE, fractional excretion.

Both ketamine and xylazine can cause a dose-dependent respiratory depression.<sup>25</sup> However, xylazine is known to cause minimal effect on respiration.<sup>26</sup> In the current experiment, a decrease in the respiratory rate that was observed following the administration of anesthetic drugs was not affected by LPS except at the 6-hour time point. Oxygen saturation was unchanged in the controls and decreased at the early time points for all LPS groups; the most important effect was seen with 1  $\mu$ g/kg and 10  $\mu$ g/kg LPS where cardiac frequency also decreased. The significant cardiac frequency increase with the high LPS dose could be an explanation for the greater oxygen saturation observed in this group. Therefore, the most important anesthetic parameter to monitor when endotoxemia is present during anesthesia would be oxygen saturation that significantly decreases with LPS, as this could very well be related to decreased cardiac output.<sup>27</sup> Ketamine is known to increase cardiac frequency, but variable effects have been seen in cardiac output in rats.<sup>26</sup> Since xylazine causes bradycardia,<sup>26</sup> the increase in plasmatic concentrations of xylazine with the low and moderate LPS concentrations could be related to the decrease in cardiac frequency.

An increase in alanine aminotransferase is commonly used to indicate hepatic damage in rat experimental models of sepsis.<sup>28,29</sup> In the current study, blood biochemistry showed no significant effect on hepatic function with LPS even though minor histological lesions were present in all LPS groups, suggesting that sufficient normal hepatic tissue is still present to preserve liver function. Even without lesions, hypoalbuminemia and hypoproteinemia are frequently observed with isoflurane or ketamine–xylazine anesthesia,<sup>10,30</sup> suggesting an effect on hepatic metabolism. Ketamine is N-demethylated by cytochrome P450 into norketamine.<sup>31</sup> It has been reported that cytochrome P3A4 is principally responsible for this metabolism in humans and cytochrome P3A in other mammals.<sup>31,32</sup> Xylazine is metabolized extensively after intramuscular injection. It has been shown that inhibitors of cytochrome

P3A4, such as chloramphenicol, alters xylazine metabolism.<sup>33</sup> Pretreatment with liver cytochrome P450 inhibitors cause a prolongation of ketamine–xylazine anesthesia in mice, rats, and chickens.<sup>34</sup> Animal studies show that LPS causes an important decrease in cytochrome P450 activity,<sup>6,7</sup> which would increase plasmatic drug concentrations metabolized by this pathway. Furthermore, LPS can cause acute renal insufficiency and indirectly affect renal excretion of drugs.<sup>35,36</sup> In the current study, blood biochemistry and histopathology showed no significant effects of LPS on renal function and therefore renal metabolism doesn't appear to have been altered. Theoretically, plasmatic concentrations of both drugs following the LPS injection should have increased in comparison with control animals receiving saline. These findings fit very well with the increased plasmatic concentrations and urinary excretion of xylazine, as a decrease in metabolism could occur with endotoxemia. However, they do not explain the decreased or unchanged AUC of ketamine even though urinary excretion increased. The urinary excretion of ketamine (fractional excretion for the 10  $\mu$ g/kg and 100  $\mu$ g/kg groups was 0.7 and 2.7, respectively, greater than the controls) was however much less pronounced than for xylazine (fractional excretion for the 10  $\mu$ g/kg and 100  $\mu$ g/kg was 26.8 and 8.5, respectively, greater than the controls). Here, again, it is suggested that a redistribution of the drugs to tissues, including the brain, may explain a prolonged anesthesia duration. A better understanding of the hepatic and renal excretion of ketamine and xylazine during endotoxemia is also required to properly interpret the results. These changes could be a consequence of systemic inflammation and therefore other inductors of inflammation should also be evaluated.

All albumin plasmatic concentrations were lower after ketamine–xylazine injection; however, the most important decrease was seen with the 100  $\mu$ g/kg LPS administration. This lower albumin concentration in the high LPS group could cause an increase in the free plasmatic drug fraction for both ketamine and xylazine, and could consequently cause a greater distribution to the central nervous system, which could explain longer anesthesia duration at least for the high LPS dose. Since protein binding of ketamine is low (50%–60%),<sup>26</sup> and no data is available for the protein binding of xylazine,<sup>26</sup> a clear conclusion regarding the effects of hypoalbuminemia in the current experiment is purely speculative.

Plasmatic pharmacokinetic parameters for ketamine cannot explain the difference in anesthesia duration since ketamine AUCs were either unchanged or decreased with

LPS administration. A decreased AUC was only seen with 10 µg/kg LPS, with a corresponding increase in the plasmatic clearance and a nonsignificant, but nearly doubled, excreted urinary fraction. Pharmacokinetic parameters for xylazine could be associated with the longer anesthesia duration since its AUC increased for both the 10 µg/kg and 100 µg/kg LPS groups. Xylazine AUCs increased and the plasmatic clearances decreased for the 10 µg/kg and 100 µg/kg LPS groups even though the urinary excreted fractions increased. Xylazine exposure is therefore increased for the high LPS concentrations and this could be associated with longer anesthesia duration. Changes in the blood–brain barrier in the presence of LPS may also be responsible for the longer anesthesia duration. P-glycoprotein is a brain-to-blood efflux system that controls the ability of many drugs and endogenous substances to access the brain. In vitro work has shown that inflammatory states mediated through LPS may stimulate P-glycoprotein activity.<sup>37</sup> Further studies evaluating the availability of ketamine and xylazine in brain tissue following LPS administration should be conducted to verify this hypothesis.

## Conclusion

LPS has a profound effect on the physiological and pharmacological parameters following an administration of ketamine and xylazine given at an anesthetic dose. Anesthesia duration significantly increased for the moderate and high LPS groups. The most important physiological change that occurred with LPS was a decrease in oxygen saturation, and for blood biochemistry a decrease of serum albumin. Ketamine pharmacokinetics were not affected except for the 10 µg/kg LPS group, where a decrease in AUC<sub>0-t</sub> and half-life and an increase in the clearance were observed. For xylazine, the AUC increased and the clearance decreased for the moderate and high LPS groups. Dose adjustments could be made to modify anesthesia when fever or endotoxemia are present in animals to obtain a more rapid recovery after anesthesia. The results suggest that the xylazine dose could be reduced since its biodistribution appears to increase with moderate and high doses of LPS.

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## Disclosure

The authors report no conflicts of interest in this work.

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