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Safety and Efficacy of Ketamine Versus Ketamine-Fentanyl-Dexmedetomidine Combination for Anesthesia and Analgesia in Rats

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Abstract

Ketamine (KET), a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, is most frequently used as an anesthetic, analgesic, and sedative drug in pediatric clinical practices. However, the adverse effects of KET administration such as psychotic episodes limited the use of KET. The aim of the present study was to evaluate whether the addition of small doses of fentanyl (FENT) and dexmedetomidine would reduce the overall KET consumption without concession on the safety and efficacy of anesthesia and analgesia in rats. We compared the effects of KET (50 mg/kg) administration alone and KET (25 mg/kg) combined with FENT (0.005 mg/kg) and dexmedetomidine (0.05 mg/kg) (KFD) on the times of onset and duration of anesthesia and analgesia. Compared with the KET group, the KFD group provides similar onset time of anesthesia, but longer duration of anesthesia, and better analgesic effect. Unlike the KET group, the KFD group had a lower heart rate and higher respiratory rate. Meanwhile, KFD induced markedly changes in the electroencephalography (EEG) spectral power when compared with control and KET. Furthermore, combination of FENT and dexmedetomidine alleviated the liver toxicity of KET. These results indicated that, when compared with KET alone, the administration of KFD combination offered safer and more efficient anesthesia.

Keywords

ketamine, fentanyl, dexmedetomidine, anesthesia, analgesia

Introduction

During surgery and other interventions, anesthesia is used to control pain, anxiety, and consciousness.[1,2] The ideal anesthesia regime should be safe and effective with rapid onset, earlier recovery, and minimal drug adverse effects. Combinations of drugs with different pharmacologic mechanisms may provide better anesthesia and analgesia effects than each individual drug given alone, exerting better anesthetic-sparing effect.[3] Ketamine (KET), a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, is one of the most frequently used anesthetics.[4,5] Ketamine produces dissociative anesthesia and potent analgesia, which is characterized by the sympathomimetic effects on the cardiovascular system (increases in the heart rate, cardiac output, and blood pressure) and minimal respiratory depression.[6,7] Due to its psychotic side effects and poor ability to provide adequate skeletal muscle relaxation,[8,9] the use of KET is limited, which can be overcome by finding a balanced safe anesthesia to reduce the doses

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required for each individual drug and still maintaining a clinical effect.

Dexmedetomidine (DEX) is a potent and short-acting α2-adrenoceptor agonist that has excellent sedative, analgesic, antisympathetic, and anesthetic-sparing characteristics.[10] Dexmedetomidine has been increasingly used as a component of general anesthesia to reduce anesthetic doses and provide reliable sedation and short-term analgesia during the perioperative period.[11,12] It generates a unique electroencephalography (EEG) mode of sleep which is similar to that of natural sleep that allows easy arousal.[13] However, DEX can cause adverse reactions such as hypotension and bradycardia.[14] Combined with the properties of DEX and KET, anesthesiologists found that DEX could prevent the hypertension, tachycardia, and emergence phenomena associated with KET. On the other hand, KET may lessen the adverse effects of DEX.[15-17]

Fentanyl (FENT) is a synthetic μ-opioid receptor agonist with 50 to 100 times the potency of morphine.[18] It is commonly used in general and regional anesthesia as a narcotic analgesic agent because of its rapid onset, short duration, and potent analgesia.[19] Akelma et al found that KET-FENT combination offers better hemodynamic properties and less side effects than KET–midazolam combination in the pediatric extracorporeal shock wave lithotripsy procedure.[20] The aim of this study was to evaluate the effect of the addition of DEX and FENT on KET consumption and compare the anesthetic, cardiorespiratory, analgesic, and recovery effects, hepatorenal and brain histopathology of KET and KET-FENT-DEX (KFD) combination in rats. We used the doses for each drug in combination based on previous studies, with modifications.[21-24]

Materials and Methods

Animals

Sprague-Dawley (SD) rats (5-6 weeks old, male) were used in this study. The animal experiments were approved by the Animal Care Committee of the Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine (Shanghai, China). All of the rats were maintained under controlled temperature with a 12-hour light/dark cycle (7 AM to 7 PM). Food and water were provided ad libitum.

Anesthesia

Ketamine (Gutian Medical Inc., Fujian, China; 50 mg/mL), FENT (Humanwell Pharmaceutical, Yichang, China; 0.05 mg/mL), and DEX (Guorui Medical Inc., Sichuan, China; 0.1 mg/mL) were dissolved in a sterile saline solution (Chimin Pharmaceutical co. Ltd., Zhejiang, China) before intraperitoneal injection at 10 mL/kg body weight. Rats were randomly divided into 3 groups (n = 5-10): saline solution (CON), 50 mg/kg KET, and a combination of 25 mg/kg KET, 0.005 mg/kg FENT, and 0.05 mg/kg DEX (KFD); the combination of drugs was administered in a single injection. Data in the literature suggest KET doses ranging from 10 to 100 mg/kg.[25,26] In pilot experiments, we found that KET 50 mg/kg alone could induce and maintain about 30 minutes of anesthesia in rats, and therefore, this dose was chosen in the present study. The doses of each drug in the combination were modified from previous studies.[21-24]

The anesthesia and analgesia responsiveness were evaluated by the absence or presence of righting reflex and toe pinch. The righting reflex was accessed by placing the rat in the supine position and observing whether it was able to turn prone. Then, a toe pinch test was performed. A warming blanket was used to maintain the rectal temperature between 37 and 38°C. The anesthesia time was divided into the following intervals: (1) the induction time is defined as the time from the anesthetic(s) administration to complete loss of the righting reflex, no response to being placed in supine position; (2) the analgesic time is defined as the time from the anesthetic(s) administration to complete no response to toe pinch; (3) the pain recovery time is defined as the time from the anesthetic(s) administration until tail flick and limbs withdrawal to pinch; (4) the righting reflex recovery time is defined as the time from the anesthetic(s) administration to awake, returns to prone position (Figure 1A).

Histopathology

Based on previous studies,[26-28] 6 hours after administration of the anesthetic(s), rats were sacrificed, and the liver, kidney, and brain were collected. The main lobe of liver, kidney, and brain was fixed in 10% neutral buffered formalin for histological examination. Tissue sections were stained with hematoxylin and eosin. The specimens were evaluated by light microscopy.

EEG Surgery and Recording

After SD rats were anesthetized with sevoflurane, they were immobilized on a pad in the stereotaxic apparatus with ear bars. Meanwhile, a mask attached to the sevoflurane anesthesia machine (4% sevoflurane supplemented with O_2 at 1 L/min) (RWD, Shenzhen, China) was fixed on the face of the rat to maintain anesthesia. On the basis of the atlas of rat brain coordinates,[29] 2 coordinates of locations of interest were determined (relative to bregma: AP [anterior/posterior] + 1.0 mm, ML [medial/lateral] + 1.0 mm, and AP − 3.0 mm, ML + 2.5 mm). The skull periosteum was removed, and then stainless screws were threaded into the skull until they touch the dura. Dental cement was used to fix the screw-lead assemblies to the skull. After the surgery, the rats recovered were singly housed in cages for 1 week with free access to food and water.

An amplifier (A-M Systems, Inc. Washington) was used to collect and amplify the EEG signals. Electroencephalography signals were recorded on a computer with the Spike Hound v1.2 software package.[30] The high-pass filter was set at 0.3 Hz, and the low-pass filter was set at 0.3 kHz. There is a dip at 50 Hz of the power spectra. We used a notch filter to remove the line (50 Hz) frequency while drafting the logarithmic power

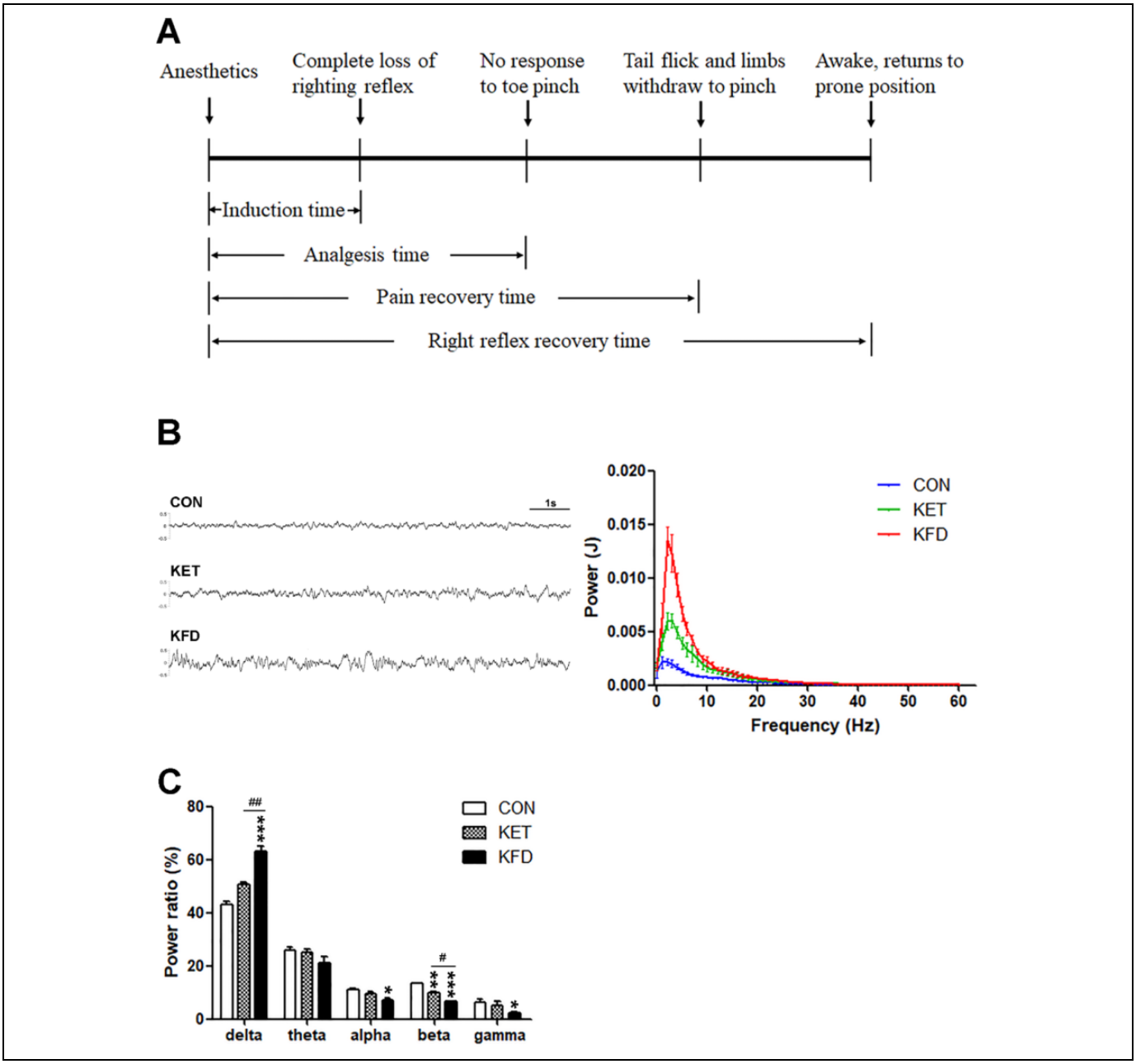

Figure 1. A, The anesthesia time was divided into 4 intervals. B, Representative EEG activity traces and corresponding power spectra recorded after saline or anesthetic(s) administration. C, Spectral analyses of EEG power showed that KFD increased δ (0.5-4 Hz) and decreased α (8-12 Hz), β (15-32 Hz), and γ (32-60 Hz) power. Data shown in the spectral power ratio analyses were mean ± SEM. Significant difference was determined by one-way ANOVA, n = 3, $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$ vs CON group. $^{\#}P < .05$, $^{\#\#}P < .01$ vs KET group. KET indicates ketamine; KFD, ketamine-fentanyl-dexmedetomidine; EEG, electroencephalography; SEM, standard error of the mean; CON, control; ANOVA, analysis of variance.

spectra of EEG. Electroencephalography data in all rats were recorded for 1 hour after administration.

Surface Electrocardiography

Once anesthetic(s) administration was completed and righting reflex was lost, the rats were placed in supine position on a Mouse Monitor S (Indus Instruments) heating pad with needle electrocardiography (ECG) leads and recorded according to the manufacturer's recommendation. Respiratory rate and SpO_2 were measured using the same equipment.

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). Student's *t*-test (two-tailed) or a one-way analysis of

Table 1. Anesthetic and Analgesic Effects in 5 to 6 W SD Rats After Injected With Anesthetics.[a]

Group	Induction Time (minutes)	Analgesic time (minutes)	Pain Recovery Time (minutes)	Righting Reflex Recovery Time (minutes)
KET	3.26 ± 0.10	—	—	23.39 ± 0.91
KFD	3.56 ± 0.15	9.86 ± 0.32	32.81 ± 0.54	59.76 ± 0.57[b]

Abbreviations: KET, ketamine; KFD, ketamine-fentanyl-dexmedetomidine; SD, Sprague-Dawley; SEM, standard error of the mean.
[a] KET: 50 mg/kg of ketamine; KFD: 25 mg/kg of ketamine, 0.005 mg/kg fentanyl, and 0.05 mg/kg of dexmedetomidine. Significant difference was determined by 2-tailed Student's *t*-tests, n = 10, mean ± SEM.
[b] $P < .001$ vs KET.

Table 2. Physiological Parameters During 30 minutes of Anesthesia.[a]

Time (minutes)	Physiological Parameters	CON	KET, 50 mg/kg	KFD
2	HR(bpm)	423.33 ± 3.34	445.67 ± 25.31	322.67 ± 4.23[b,c]
	RR(bpm)	56 ± 13.06	36.33 ± 2.6	67.67 ± 2.6
	SpO_2(%)	100 ± 0.00	92.83 ± 1.58[b]	96.1 ± 0.78
5	HR(bpm)	430.67 ± 3.14	469.67 ± 3.66[d]	323 ± 1.7[d,e]
	RR(bpm)	56.33 ± 12.53	40.67 ± 5.19	64.33 ± 0.27
	SpO_2(%)	98.93 ± 0.87	94 ± 2.83	97.47 ± 1.04
10	HR(bpm)	438.67 ± 1.36	459.33 ± 4.23[b]	318.67 ± 1.96[d,e]
	RR(bpm)	41.33 ± 5.19	36.67 ± 2.76	64.33 ± 0.54[b,c]
	SpO_2(%)	99.1 ± 0.54	92.67 ± 2.49[f]	96.13 ± 0.28
15	HR(bpm)	445.67 ± 1.96	451.67 ± 9.66	313.33 ± 2.60[d,e]
	RR(bpm)	41.67 ± 0.27	48.33 ± 2.99	65 ± 0.00[c,d]
	SpO_2(%)	98.93 ± 0.52	97.57 ± 1.99	97.8 ± 0.66
20	HR(bpm)	436 ± 2.94	458.67 ± 11.94	309.33 ± 2.6[d,e]
	RR(bpm)	40 ± 0.47	41.33 ± 0.27	64 ± 0.47[d,e]
	SpO_2(%)	98.53 ± 0.4	93.2 ± 2.7	97.37 ± 1.11
30	HR(bpm)	436.33 ± 2.6	472 ± 20.07	314.67 ± 4.72[c,e]
	RR(bpm)	41 ± 0.47	40.67 ± 5.19	60 ± 3.3[f,g]
	SpO_2(%)	98.23 ± 1.06	98.03 ± 1.61	98.2 ± 1.39

Abbreviations: KET, ketamine; KFD, ketamine-fentanyl-dexmedetomidine; CON, control; SEM, standard error of the mean.
[a]KET: 50 mg/kg of ketamine; KFD: 25 mg/kg of ketamine, 0.005 mg/kg fentanyl, and 0.05 mg/kg dexmedetomidine; CON: saline injection. Significant difference was determined by one-way analysis of variance, n = 6, mean ± SEM.
[b]$P < .01$.
[c]$P < .01$.
[d]$P < .001$ vs CON.
[e]$P < .001$ vs KET.
[f]$P < .05$.
[g]$P < .05$.

variance was used to analyze data unless otherwise mentioned. All statistical analyses were performed using SPSS v.11.5 software (SPSS, Chicago, Illinois). A *P*-value of less than .05 was considered statistically significant.

Results

Anesthesia

The anesthesia onset was evaluated by the absence of righting reflex and toe pinch. The anesthesia induction time was similar between KET and KFD groups. However, unlike KFD group that had analgesic effect, there was no analgesic effect in KET group, which may be associated with the subanesthetic dose (50 mg/kg) we used. Moreover, the time to recovery of the righting reflex was significantly longer in the KFD group (59.76 ± 0.57 minutes) when compared with the KET group (23.39 ± 0.91 minutes, $P < .001$) (Table 1).

Cardiovascular and Respiratory System Effects

SpO_2, heart rate, and respiratory rate were measured to estimate differences at each time point over a 30-minute anesthesia duration. The KFD group had a significantly lower heart rate compared with the control and KET groups during the course of detection, and the KFD group had a higher respiratory rate than the control and KET groups at several time points (10, 15, 20, and 30 minutes). Meanwhile, when compared with the control group, the KET group had a higher heart rate at 5 and 10 minutes time points. Although KET slightly decreased respiratory rate, there were no significantly differences between the control and KET groups. Moreover, the KET group had a lower SpO_2 than the control group at 2- and 10-minute time points (Table 2).

Electroencephalography

Electroencephalography spectral power was recorded during the anesthesia period. The spectral analyses of EEG data

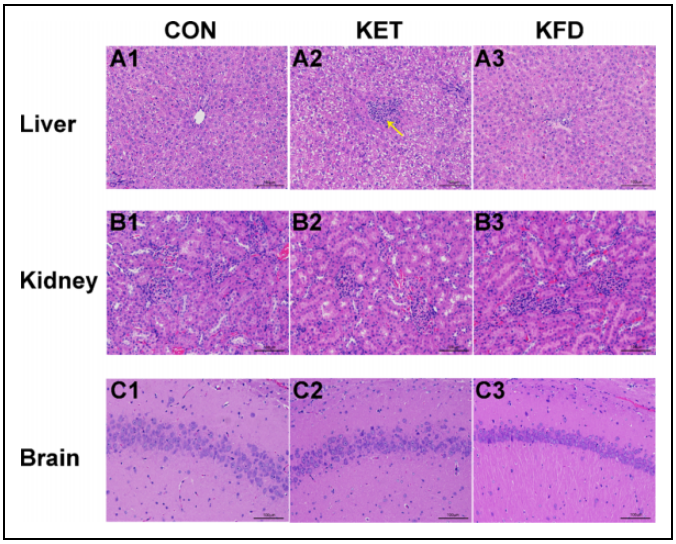

Figure 2. Six hours after anesthetic administration, rats (n = 5) were sacrificed. Then, the livers, kidneys, and brain were collected and stained with HE (×40). A, Yellow arrow: inflammatory cells. B, In kidneys, there were no particular change in the KET and KFD groups when compared with the control group. C, There was no apparent difference in brain tissue between groups. KET indicates ketamine; KFD, ketamine-fentanyl-dexmedetomidine; HE, hematoxylin and eosin.

showed that slow waves (δ: 0.5-4 Hz) had the highest power after anesthetic administration (Figure 1B and C). Compared with the the control and KET groups, the KFD group had higher power ratio of the δ frequency band (CON: 43.17 ± 1.04%; KET: 50.71 ± 0.78%; KFD: 62.95 ± 1.87%) and lower power ratio of the α band (8-12 Hz; CON: 10.90 ± 0.51%; KET: 9.49 ± 0.54%; KFD: 7.14 ± 0.74%), β band (15-32 Hz; CON: 13.46 ± 0.14%; KET: 9.90 ± 0.42%; KFD: 6.5 ± 0.23%), and γ band (32-60 Hz; CON: 6.42 ± 0.78%; KET: 4.90 ± 1.32%; KFD: 2.27 ± 0.21%). There was no significance difference in the power ratio of θ band (4-8 Hz; CON: 26.05 ± 0.71%; KET: 25.00 ± 0.90%; KFD: 21.07 ± 1.84%) between groups (Figure 1B and C). These results indicated that KFD combination provided deeper anesthesia than KET alone.

Histopathology Examination

It has been reported that chronic or repeated administration of KET may induce hepatotoxicity,[31] renal failure,[32] and cell apoptosis in brain.[33,34] However, the effect of a single dose of KET on neuroapoptosis in brain is controversial.[28,35] Moreover, liver and kidney are the main organs for KET metabolism and clearance, respectively.[36,37] Hence based on the important roles of these 3 organs, we determined whether a single injection of KET or KFD could induce the toxicity of these tissues, and further compared the effects of KET and KFD on these tissues in this current study. As shown in Figure 2A, compared with the control group, hepatocytes in the KET group showed obviously focal inflammatory cell infiltration in the lobule (yellow arrow), while KFD group showed no distinct injury. Furthermore, there were no treatment-related morphologic changes in the kidneys and brains between groups (Figure 2B and C).

Discussion

During surgery, different anesthesia regimes can be used to allay anxiety and to decrease pain in patients or laboratory animals. Since a given anesthesia regime may impact the results of surgery or experiment, the choice of a suitable anesthesia should be considered carefully. Ketamine is a widely used pediatric anesthetic that primarily blocks NMDA receptors.[38,39] As it provides both unconsciousness, analgesia, and amnesia, it is the single agent possessing the most properties of a complete anesthetic. However, KET is a direct myocardial depressant, and like other adverse reactions, this side effect profile also limits its ubiquitous use. To reduce the adverse effects of each individual anesthetic, anesthesiologists usually use a rational combination of anesthetic agents. Dexmedetomidine is a highly selective α2 agonist that has been developed for human clinical use as an anesthetic and sedative. It provides potential advantages of anxiolysis and analgesia without respiratory depression. In recent years, as an anesthetic adjuvant during operation, it has been investigated and found effective in both clinical studies[40] and animal models.[41,42] Fentanyl, an opioid analgesic, works primarily at the μ-receptor and is commonly used as an adjunct in neuroleptoanalgesia. Lipid soluble FENT was fast acting, beginning to provide analgesia in 1-2 minutes after intravenous administration. Considering the different mechanisms of the 3 drugs, we chose DEX and FENT to combine with KET to achieve a balanced anesthesia in this study.

The current work showed that KET alone or combination with DEX and FENT led to comparable levels of anesthesia onset (Table 1). Although there were no apparent differences between groups in the loss of righting reflex, only the lost toe pinch reflex was found in the KFD group. Meanwhile, the time to return of the righting reflex was significantly longer in the KFD group when compared with the KET group, which indicated that KFD may be a good choice for long-term surgery. It is reported that KET may stimulate the cardiovascular system and cause minimal respiratory depression. Consistent with the previous reports, our results demonstrated that KET alone obviously increased heart rate and slightly decreased respiratory rate of rats. However, KFD administration led to a lower heart rate and higher respiratory rate. Furthermore, the KET group had a lower SpO_2 than the control group, while the KFD group had no effects on the SpO_2 of rats (Table 2). On the whole, these results are in accordance with those found in the literature. The KFD combination provides a stable anesthesia and alleviates the tachycardia effect caused by KET.[16,20,43] Moreover, the use of DEX and FENT reduces anesthetic requirements.

Given that KFD delayed recovery from anesthesia compared with KET, we further measured EEG spectral power of

rats to test EEG changes. The results of this study showed that KFD induced markedly changes in the EEG and induced deeper anesthesia (Figure 1B and C). Although previous studies have indicated that long-term or repeated administration of KET may induce liver, kidney, and brain damage,[31-33] whether a single injection of KET could induce injury of these tissues is unclear. Hence, in the present study, we detected the effects of KFD and KET administered in a single injection on the morphology of these 3 organs. Compared with the control group, KET alone induced focal inflammatory cell infiltration in the lobule, while the KFD group showed no obvious injury (Figure 2A). Moreover, both KET and KFD groups had no treatment-related histopathology changes in the kidneys and brains of rats (Figure 2B and C).

In this current study, although we propose a new anesthesia regime consisting of KET, DEX, and FENT in rats, to further clarify the effects and doses of KFD in human, more studies are needed in the future. In conclusion, the present study demonstrated that low dosage of KET combined with DEX and FENT led to comparable levels of anesthesia onset and even better analgesic effects to that of high dosage of KET alone administration. Despite KFD leading to lower heart rate and higher respiratory rate, it had no effect on the liver, kidney, and brain of rats when compared with control and KET groups. Therefore, our results suggest that the administration of KFD combination provides safer and more efficient anesthesia than that of KET alone.

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Author contribution

Chunzhu Li and Jiali Peng contributed equally to this work.

Declaration of Conflicting Interests

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