Original Article

Safety and Efficacy of Ketamine-Fentanyl-Dexmedetomidine-Induced Anesthesia and Analgesia in Neonatal and Aged Rats

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Abstract

The efficiency of many anesthetic regimens is controversial, with side effects especially in the vulnerable children and old population. The study aimed to evaluate the safety and efficacy of low-dose combination of ketamine, fentanyl, and dexmedetomidine (KFD) for anesthesia and analgesia in the neonatal and elderly rats. KFD rapidly induced anesthesia and analgesia in either postnatal days 6 (P6) or 13 months (13M) old rats. Meanwhile, KFD administration had no adverse effects on the cardiovascular and respiratory systems. Compared with control group, there were no distinct morphologic changes in kidney, liver, and brain in KFD group. Moreover, administration of KFD had no influence on hepatic and renal function in rats of both ages. Furthermore, there was no obvious difference in cognitive function between control and KFD groups. These results indicated that the administration of KFD combination offered safe and efficient anesthesia. Collectively, our results suggest the potential implication of the KFD combination in anesthesia management.

Keywords

anesthetics, anesthesia, analgesia, neonatal, aged

Introduction

Anesthetics are frequently used in surgery and interventional procedures for people of all ages. Amounts of neonates and young children are exposed to anesthesia every year.[1] Meanwhile, as the global population ages rapidly, the number of elderly patients undergoing surgery is also increasing.[2,3] The developing and aging brain may be vulnerable to anesthesia.[4] Animal investigations suggest the potential "double-edged" sword of anesthetics administration in the young; these anesthetics may have neuroprotective effects in certain circumstances, but can be neurotoxic in others.[5] Furthermore, in recent decades, growing evidence shows that multiple or prolonged exposure to general anesthesia in early infancy has the potentials for long-lasting behavioral deficits later in life.[6,7] Moreover, it has been demonstrated that delirium and postoperative cognitive dysfunction often occur in older people after anesthesia/surgery.[8-10] Physiology, as well as the pharmacokinetics and pharmacodynamics of drugs, change with age, and these may affect the anesthetic management, adverse reactions, postoperative recovery, and outcomes. A balance must be struck between the potential toxicity and the importance of providing adequate anesthesia. Therefore, it is critical to ensure that anesthesia regimes are safe and effective in neonates, children, and older patients.

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Ketamine is a noncompetitive NMDA receptor antagonist that is widely used to induce anesthesia and analgesia in clinic.[11] However, ketamine can cause undesirable adverse effects, including emergence agitation and increased blood pressure.[12] And it was found that ketamine administered to pregnant rats in the second trimester causes long-lasting behavioral disorders in offspring.[13] Dexmedetomidine, a highly selective and short-acting α2-adrenoreceptor agonist, is used for sedation and analgesia in clinical practice and shows an anesthetic-sparing effect.[14,15] It has been reported that dexmedetomidine can attenuate isoflurane-induced neurocognitive impairment in neonatal rats.[16] And Wang et al[17] found that dexmedetomidine can protect the immune function, and relieve perioperative stress and inflammation of surgical patients, all of which may help to reduce postoperative complications and improve clinical outcomes. Studies have shown that combination of dexmedetomidine and subanesthetic doses of ketamine provides effective sedation and analgesia in clinical setting and reduces each other's side effects.[18,19] Fentanyl, a full agonist with high selectivity for the μ-opioid receptor, is often used to relieve anxiety and to alleviate pain associated with surgery.[20] It is characterized by fast onset and short duration.[21] Due to pharmacokinetic features and adverse effects of anesthetics, they are not administered alone but often combined with other anesthetics. The combination of drugs with different pharmacological mechanisms may provide greater anesthesia and analgesia effect than each drug given alone, with further anesthetic-sparing effect.[22-24] In previous studies, we showed that the low-dose combination of ketamine, fentanyl, and dexmedetomidine (KFD) offered safe and efficient anesthesia in adults.[25,26] However, it is unknown whether the KFD combination is safe and effective for neonates and the elderly.

In consideration of age-related differences in pharmacokinetics and pharmacodynamics of anesthetic drugs, the present study was undertaken to investigate the anesthetic, analgesic, and physiological effects of KFD (ketamine, 10 mg/kg; fentanyl, .01 mg/kg; dexmedetomidine, .1 mg/kg) combination; the effects of administration of KFD combination on histomorphology and clinical biochemistry values of kidney, liver, and brain; and the cognitive function after treatment of KFD combination in neonatal and aged rats.

Methods

Animals

Sprague-Dawley rats (postnatal day 6 or 13 months 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), and all methods were performed in accordance with the relevant guidelines. Rats were maintained in a temperature-controlled environment under a 12 h light/dark cycle (07:00 AM to 07:00 PM). Food and water were provided ad libitum.

Anesthesia

Ketamine (Gutian Medical, Inc., Fujian, China; 50 mg/mL), dexmedetomidine (Guorui Medical, Inc., Sichuan, China; .1 mg/mL), and fentanyl (Humanwell Pharmaceutical, Yichang, China; .05 mg/mL) were dissolved in a sterile saline solution (Chimin Pharmaceutical Co., Ltd., Zhejiang, China) before intraperitoneal injection (i.p.) at 10 mL/kg body weight. The drug doses were selected on the basis of previous studies with slight modifications.[25,26]

The anesthetic and analgesic effects were evaluated by the duration of the ketamine-induced loss of righting reflex (LORR) and the absence of the pain reflex, and we tested these indicators as previously reported.[21] The anesthesia time was divided into the following intervals: (1) The induction time defined as the time from the anesthetics administration to complete LORR; (2) The analgesia onset time defined as the time from the anesthetics administration to complete no response to toe pinch; (3) The duration of analgesia time defined as the time from the absence of the limb withdrawal reflex to the return of the tail flick and limb withdrawal reflex; (4) The duration of LORR (Fig. 1A).

Histopathology

Six hours after administration of the anesthetics, rats were sacrificed and kidney, liver, and brain were collected as previously described.[25] The main lobe of kidney, liver, and brain were fixed in 10% neutral buffered formalin for histological examination. Tissue sections were stained with hematoxylin and eosin (HE). The specimens were evaluated by light microscopy.

Serological Analysis

Rats were anesthetized with isoflurane, and blood was collected from the fundus vein of each animal before anesthetics administration (0 h) and at .5, 12, 24, and 48 h after anesthetics injection. Then, the blood samples were centrifuged at 4°C and 4000 g for 10 min to obtain 200 μL of serum for testing. Serum alanine aminotransferase (ALT), aspartate transaminase (AST), creatinine (CREA), and urea were determined using an automatic Hitachi Clinical Analyzer Model 7080 (Hitachi High-Technologies Corporation, Tokyo, Japan).

Surface Electrocardiography (ECG)

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

Morris Water Maze

One month after saline or KFD administration, rats received Morris water maze (MWM) test for spatial learning and memory abilities. The MWM apparatus included a black circular pool with the following dimensions: 160 cm in diameter and 50 cm in height and filled with warm water at

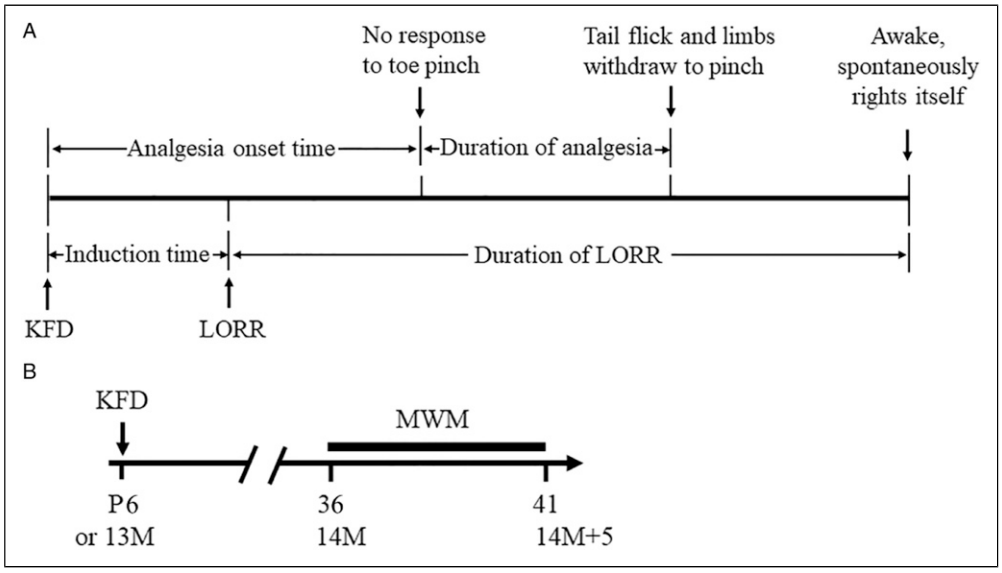

Figure 1. The schematic diagram of the evaluation of anesthesia and MWM experiment design. (A) The anesthesia time was divided into 4 intervals. After rats were anesthetized, the duration of LORR and analgesia were recorded. (B) Design of MWM experiment. KFD: combination of 10 mg/kg ketamine, .01 mg/kg fentanyl, and .1 mg/kg dexmedetomidine; the combination of anesthetics was administered in a single injection.

23°C. A 10-cm-diameter platform was submerged 2 cm below the water surface. The acquisition phase included 3 trials each day for 4 consecutive days. During each trial, the rats were released into the water from a specific starting point located at the quadrant opposite the platform. The time to find the platform and the distance swum before reaching the platform were recorded, and the rats were allowed to swim freely until they reached the platform in 2 min and stayed on it for 30 s. If the rats did not locate the platform, they were gently guided to the platform and were allowed to rest on it for 30 s, and the latency was recorded as 120 s. On the fifth day, the rats were tested on a spatial probe trial in which the platform was removed, and they were allowed to swim freely for 120 s (Fig. 1B). The time to reach the platform initially, the ratio of time spent in the range around the platform as determined by software, and the number of times that the rats crossed the platform was recorded.

Statistical Analysis

Data are presented as the mean ± SEM (standard deviation). Student's *t*-test (2-tailed) was employed to analyze data unless otherwise mentioned. All statistical analyses were performed using SPSS v.11.5 software (SPSS, Chicago, IL, USA). A P-value of less than .05 was considered statistically significant.

Results

Anesthetic and Analgesic Effects of KFD in P6 and 13M Rats

After intraperitoneal injection of KFD, the anesthetic and analgesic effects were recorded. As shown in Table 1, treatment with KFD rapidly induced LORR in P6 (1.51 ± .05 min) and 13M (4.95 ± .17 min) rats. The analgesia onset time of KFD in P6 and 13M rats were 4.07 ± .39 min and 8.33 ± .32 min, respectively. We found that the analgesic duration of KFD was longer in 13M (112.08 ± 4.75 min) rats than in the P6 rats (49.30 ± .21 min), and the duration of LORR was similar in both ages (P6, 172.34 ± .82 min; 13M, 193.12 ± 5.88 min).

KFD had No Obvious Adverse Effects on the Cardiovascular and Respiratory Systems

In a previous study, we found no adverse effects of KFD on the cardiovascular and respiratory systems in adult rats.[25] However, it is unclear whether KFD administration affects these systems in neonatal and old rats. Therefore, in this study, we determined the effects of KFD treatment on the cardiovascular and respiratory systems of P6 and 13M rats. Heart rates, respiratory rates and SpO_2 were detected during 150 min of anesthesia. We found that the heart rate of the both ages of rats in KFD group decreased during anesthesia compared with the control group (Tables 2 and 3), consistent with our previous study in adult rats.[20] Meanwhile, there was no significant difference in respiratory rate and SpO_2 between KFD and control groups. The results indicated that KFD administration did not cause adverse cardiovascular and respiratory reactions in P6 and 13M rats.

Histopathology Examination

Given the potential toxicity of anesthetics to kidney, liver, and brain,[27-30] we investigated the toxicity of a single injection of

Table 1. Anesthetic and Analgesic Effects in P6 or 13M SD Rats after Injected with KFD.

Age	Induction Time (min)	Analgesia Onset Time (min)	Duration of Analgesia (min)	Duration of LORR (min)
P6	1.51 ± .05	4.07 ± .39	49.30 ± .21	172.34 ± .82
13M	4.95 ± .17	8.33 ± .32	112.08 ± 4.75	193.12 ± 5.88

KFD: 10 mg/kg of ketamine, .01 mg/kg fentanyl, and .1 mg/kg of dexmedetomidine. The data were presented as mean ± SEM, n = 3-6.

Table 2. Physiological Parameters during 30 min of Anesthesia in P6 Rats.

Time (min)	PhysiologicalParameters	CON	KFD
2	HR (brpm)	331.00 ± 3.40	272.00 ± 1.25***
	RR (bpm)	60.67 ± 3.14	52.33 ± .27
	SpO_2 (%)	91.67 ± .69	94.40 ± .81
5	HR (brpm)	332.33 ± 2.18	269.33 ± .72***
	RR (bpm)	59.33 ± 6.82	48.67 ± 2.72
	SpO_2 (%)	89.90 ± 1.75	91.63 ± 1.78
10	HR (brpm)	343.00 ± 6.98	263.00 ± 1.41***
	RR (bpm)	56.33 ± 3.14	60.33 ± 10.79
	SpO_2 (%)	89.53 ± 1.85	94.07 ± 2.43
15	HR (brpm)	349.00 ± 11.84	254.00 ± 3.30**
	RR (bpm)	64.00 ± .47	52.33 ± 6.13
	SpO_2 (%)	90.40 ± 1.40	93.37 ± 2.36
20	HR (brpm)	327.33 ± 8.28	251.33 ± 5.17**
	RR (bpm)	56.00 ± 3.74	51.00 ± .00
	SpO_2 (%)	92.03 ± 1.14	92.33 ± 1.58
30	HR (brpm)	337.00 ± 2.49	254.00 ± 4.72***
	RR (bpm)	59.33 ± 2.99	55.00 ± 3.27
	SpO_2 (%)	90.97 ± 1.58	91.93 ± .50

KFD, 10 mg/kg of ketamine, .01 mg/kg fentanyl, and .1 mg/kg of dexmedetomidine; CON, saline injection; HR, heart rate; RR, respiratory rate. The data were presented as mean ± SEM, n = 6.
**P < .01, ***P < .001 vs CON.

KFD to these tissues in P6 and 13M rats. As shown in Fig. 2, KFD administration did not cause histomorphologic changes in kidney, liver, and brain compared with the control group.

Hepatic and Renal Function Examination

Considering that liver and kidney are the major organs for drug metabolism and clearance, we further examined the effects of KFD combination on these 2 tissues. Since P6 rats are not suitable for blood collection at multiple time points, serological analysis was performed on 13M rats only. As shown in Fig. 3, the serum levels of ALT, AST, urea, and CREA in KFD group were not notably different from those in control groups. These results revealed that KFD combination had no significant effect on hepatic and renal function.

KFD had No Influence on Cognitive Function

Emerging evidence suggests that anesthesia can induce cognitive dysfunction in neonates and older patients.[7,31] Thus, we further determined the effects of KFD combination on learning and memory skills in P6 and 13M rats. We performed MWM test on the rats to assess their learning and memory abilities 1 month after the drug treatment. We found that a single exposure to KFD anesthesia at P6 or 13M had no obvious effect on rats' cognitive function at P36 (Fig. 4A-E) or 14M (Fig. 4F-J), respectively. There was no apparent difference in the latency to find the platform and the swim distance between the KFD and control groups (Fig. 4, A, B, F, and G). The number of attempts to across the platform or the ratio of time spent in the range around the platform on the testing day of the KFD group was similar to those of the control group (Fig. 4, C, D, H, and I). Meanwhile, representative traces of the paths swum by the rats in the spatial probe test are also shown, with the platform encircled in blue and the range around the platform marked in orange (Fig. 4E and J). These results revealed that KFD did not affect the cognitive function of the rats at the tested age.

Discussion

The advent of anesthesia made it possible for the development of complex surgery in patients of all age groups. Notably, children are exposed to anesthetics for far more than surgical

Table 3. Physiological Parameters during 30 min of Anesthesia in 13M Rats.

Time (min)	Physiological Parameters	CON	KFD
2	HR (brpm)	410.67 ± 2.84	281.33 ± 7.15***
	RR (bpm)	48.00 ± 2.87	48.67 ± 5.44
	SpO_2 (%)	99.63 ± .30	100.00 ± .00
5	HR (brpm)	409.33 ± 2.76	274.67 ± 6.13***
	RR (bpm)	44.00 ± 2.45	48.33 ± 3.41
	SpO_2 (%)	99.67 ± .27	99.80 ± .08
10	HR (brpm)	414.67 ± 2.72	270.33 ± 5.68***
	RR (bpm)	51.33 ± .27	52.00 ± 5.44
	SpO_2 (%)	100.00 ± .00	100.00 ± .00
15	HR (brpm)	414.67 ± 1.96	265.33 ± 4.72***
	RR (bpm)	44.67 ± 2.60	52.00 ± 5.44
	SpO_2 (%)	99.67 ± .27	99.90 ± .08
20	HR (brpm)	413.67 ± 3.21	259.33 ± 4.46***
	RR (bpm)	47.67 ± 2.72	52.00 ± .47
	SpO_2 (%)	99.77 ± .19	99.70 ± .24
30	HR (brpm)	418.00 ± 2.49	249.33 ± 1.66***
	RR (bpm)	48.00 ± 2.87	52.33 ± .47
	SpO_2 (%)	99.67 ± .27	99.87 ± .11

KFD, 10 mg/kg of ketamine, .01 mg/kg fentanyl, and .1 mg/kg of dexmedetomidine; CON, saline injection. The data were presented as mean ± SEM, n = 6. HR, heart rate; RR, respiratory rate.
***P<.001 vs CON.

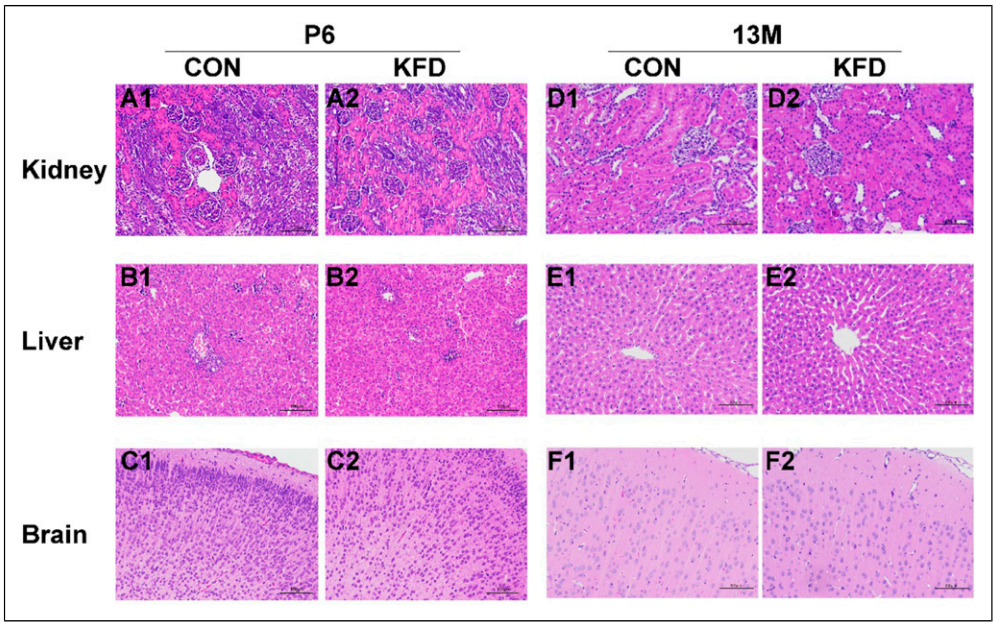

Figure 2. Six hours after anesthetic administration, rats (n = 5) were sacrificed. Then the kidneys (A and D), livers (B and E), and brain (C and F) were collected and stained with HE (×40).

procedures. Because neonates and young children lack the ability to fully self-regulate or understand the norms of the situation, they have difficulty cooperating during minimally invasive operation or radiological imaging procedures. Moreover, as the global population is aging, the number of older patients undergoing surgery is also increasing.[32,33] Due to age-related changes in physiology, pharmacokinetics and pharmacodynamics may influence the anesthetic efficacy and side effects, special attention is required for anesthetic management of young and older patients.

Ketamine is a dissociative anesthetic with potent analgesic properties, which is widely used in pediatrics.[34] Fentanyl is a short-acting synthetic opioid used worldwide. It produces analgesia by binding to opioid receptors.[35] Dexmedetomine, a

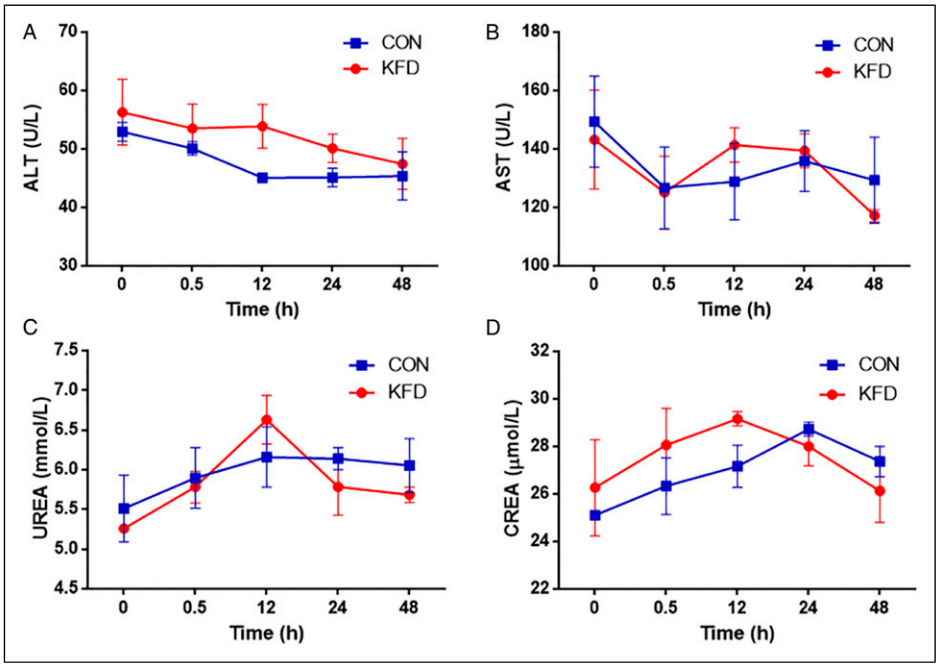

Figure 3. The detection of serum markers levels. At 0, .5, 12, 24, and 48 h after injection with KFD, the levels of serum ALT (A), AST (B), UREA (C), and CREA (D) were assessed. The data were presented as mean ± SEM, n = 3.

highly selective α2-adrenoceptor agonist, has been increasingly used as a general anesthetic adjuvant.[36] In the present study, we demonstrated that the low-dose combination of KFD is a safe and effective anesthetic formula for neonatal and old rats. The drug doses were selected on the basis of previous studies with slight modifications.[25,26] And in pilot experiments, we found that the combined doses of KFD used in our study were the minimum doses required to maintain stable anesthesia for more than 30 min in P6 rats. Additionally, the doses also had a good anesthetic effect on the elderly rats, so we chose the same doses for the experiments on the aged rats. Collectively, we found that KFD combination rapidly induced anesthesia and analgesia in rats of both ages, and the duration of LORR and analgesia of KFD was long, which was consistent with our previous study.[25,26] The data indicated that KFD may be a good option for long-term operation.

In consideration of the potential effects of anesthetics on the cardiovascular and respiratory systems,[37,38] we tested the heart and respiratory rates, and SpO_2 of neonatal and old rats after KFD treatment. Compared with the control group, administration of KFD led to a lower heart rate during anesthesia. Moreover, the respiratory rate and SpO_2 of KFD group were similar with the control group. Since anesthetics may be toxic to the kidney, liver, and brain,[39,40] we further examined the morphological changes of these tissues after KFD administration. The results of this study showed that KFD had no influence on the histomorphology of these tissues in neonatal and old rats. Furthermore, serological analysis showed no significant differences in ALT, AST, urea, and CREA levels between KFD and control groups, suggesting that KFD combination had no effect on hepatic and renal function.

Previous studies have indicated that prolonged exposure to general anesthetics may affect neurodevelopment in neonatal rats.[40] What's more, cognitive decline is common in the elderly after anesthesia and surgery.[41,42] Therefore, we performed MWM test on the neonatal and old rats to detect their learning and memory skills 1 month after the KFD treatment. We found that the KFD groups performed similarly compared with the control groups in rats of both ages. These results indicated that KFD combination did not affect brain function. Although in vivo results showed that the combination of KFD was safe and effective, our study still had some experimental limitations, such as the absence of investigations on cell injuries at cellular or sub-cellular level of vital organs and the absence of measurements of blood gases and blood pressure due to technique challenges. In consideration of reports and our previous study suggesting that there may be no gender difference in the effect of anesthetics,[26,43] male animals were selected for this study. In addition, the anesthetics we selected are all commonly used in the clinical practice, and their doses in the combination were significantly lower than those used alone. However, more studies are needed in the future to further clarify the effects and dosages of KFD in human.

In conclusion, our study demonstrated that low-dose combination of KFD provided stable anesthesia in neonatal and old rats and may be suitable for prolonged procedures. KFD treatment did not impact the cardiovascular and respiratory systems. Additionally, administration of KFD had no influence on the renal, hepatic, and cognitive functions of rats

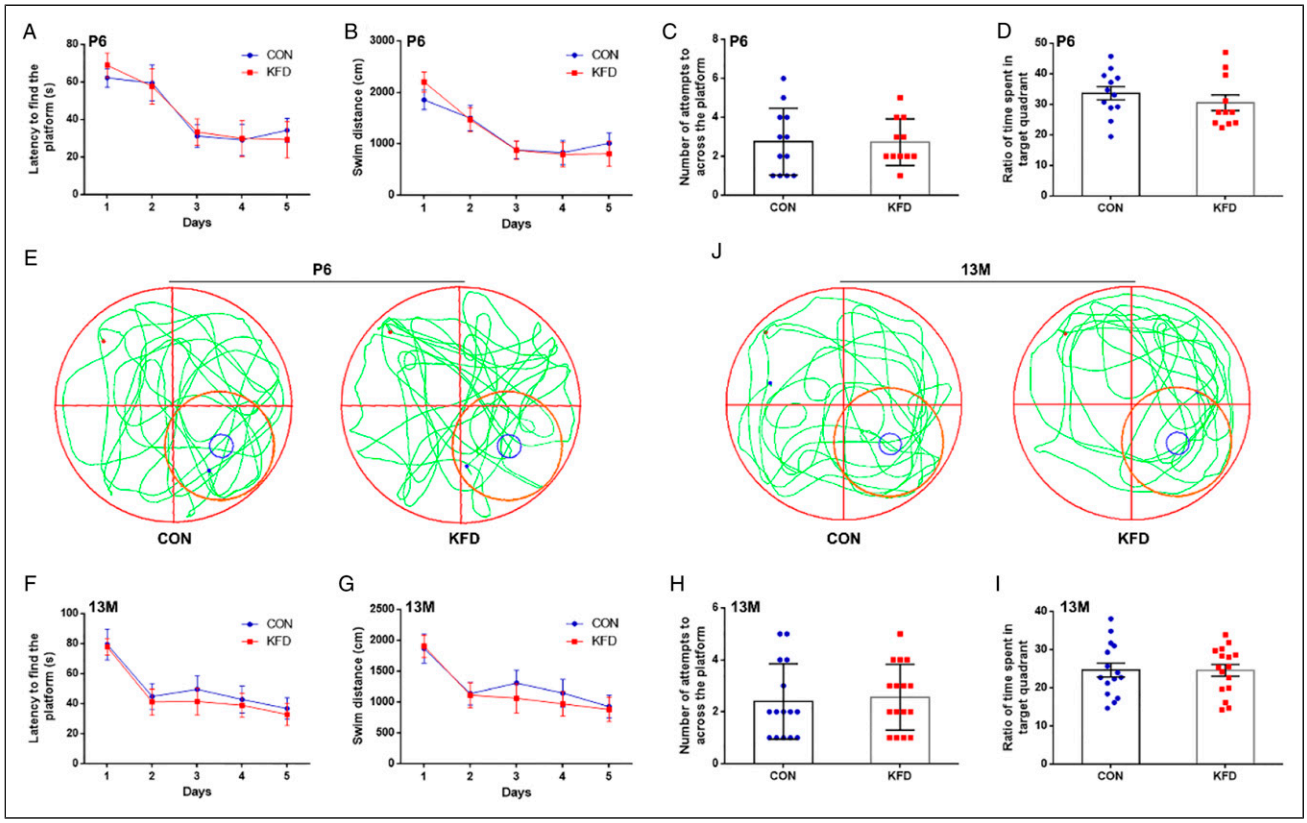

Figure 4. KFD had no effect on the cognitive function of neonatal and old rats. One month after P6 or 13M rats treated with KFD combination, the MWM test was performed. There were no differences in the latency to find the platform (A and F) and swim distance (B and G) between KFD and control group. The number of attempts to across the platform (C and H) and ratio of time spent in the range around the platform (D and I) of the KFD group were similar with the control group. (E and J) Representative traces of the movement of the rats in the spatial probe test. The blue circle represents the removed platform, and the orange represents the range around the platform. The data were presented as mean ± SEM. n = 12-16.

at either age. These results suggested that KFD combination was a safe and effective anesthetic formulation for neonatal and elderly rats.

Author Contributions

H. Jiang and C. Li designed the project.
X. Zhou and W. Li wrote the manuscript and revision with H. Jiang and C. Li
X. Zhou, W. Li and H. Wang contributed to the experiment performance
X. Zhou, W. Li and C. Li helped to analyze the data
All authors have given their final approval for the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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