Preliminary Evaluation of the Efficacy and Safety of Brimonidine for General Anesthesia

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Abstract

Background:

To determine the hypnotic and analgesic effects of brimonidine, and evaluate its efficacy and safety for general anesthesia. Potentiation of pentobarbital sleeping time with brimonidine was observed in mice, as was analgesic activity of brimonidine.

Methods:

The median effective dose (ED_{50}) and lethal dose (LD_{50}) of intraperitoneally injected brimonidine were determined in hypnotized mice. In addition, LD_{50} of intravenously injected brimonidine, ED_{50} of intravenously, intramuscularly and intrarectally injected brimonidine in hypnotized rabbits were determined. The synergistic anesthetic effect of brimonidine and chloral hydrate on rabbits was evaluated.

Results:

Intraperitoneal injection of 10 mg/kg brimonidine enhanced the hypnotic effect of a threshold dose of pentobarbital. Intraperitoneal injection brimonidine produced dose-related analgesic effects in mice. ED_{50} of intraperitoneally administered brimonidine in hypnotized mice was 75.7 mg/kg, and LD_{50} was 379 mg/kg. The ED_{50} of intravenous, intramuscular and intrarectal brimonidine for hypnosis in rabbits were 5.2 mg/kg, 8.8 mg/kg and 8.7 mg/kg, respectively, and LD_{50} of intravenous brimonidine was 146 mg/kg. Combined intravenous administration of 0.6 mg/kg brimonidine and 0.03 g/kg chloral hydrate had a synergistic anesthetic effects.

Conclusions:

Brimonidine elicited hypnotic and analgesic effects after systemic administration, and exhibited safety. Brimonidine enhanced the effects of other types of narcotics when combined.

Background

General anesthesia involves drugs that widely inhibit the central nervous system to temporarily inhibit consciousness and nerve response activities, such that the body has no response or memory to pain and noxious stimulation. Such effects may be accompanied by a decrease of muscle tension caused by central mechanisms, but the important central functional state of the medulla oblongata is maintained.

α_2 Adrenergic receptor agonists, such as dexmedetomidine, are often used as adjuncts to clinical general anesthesia, but can also be used as a basic anesthetic in combination with local anesthetics for surgery. Such anesthetics act as both sedatives and analgesics, and may have hypnotic, anti-anxiety, anti-sympathetic, and muscle relaxation effects. Moreover, these anesthetics protect organs, have good safety

profiles, are not addictive, and can enhance the functions of other central anesthetics. α_2 adrenergic receptor agonists are often used in combination with other anesthetics to meet the anesthesia needs of various surgical settings during the induction period, maintenance period, and awakening period.

In this study, we investigated the general anesthesia effects of brimonidine, a highly selective α_2 receptor agonist with high activity and good water-fat solubility, and preliminarily evaluated its safety. At present, no reports describe induction or maintenance of general anesthesia by brimonidine alone or in combination. This study evaluated the analgesic and hypnotic potentiation of brimonidine systemic administration against various animal experimental models. Here, we report the first investigation into the general anesthesia activities of brimonidine.

Methods

Laboratory Animals

Japanese white rabbits of ordinary grade (Longan Experimental Animal Breeding Center, Beijing, China), weighing 2.3–2.6 kg were used in experiments [Animal Production License No. SCXK (Beijing) 2019–0006]. In addition, specific-pathogen-free Kunming(KM) mice (Beijing Weitonglihua Experimental Animal Center, China), weighing 20.0–23.0 g were used [Animal Production License No. SCXK (Beijing) 2016–0006]. Animal experiments were conducted in the Animal Room of Tianjin Institute of Medical & Pharmaceutical Science. Permission and approval for animal studies were obtained from the Animal Ethics Committee of Tianjin Institute of Medical & Pharmaceutical Science (Approval No. IMPS-EAEP-Q-2020-01).

Chemical and Reagents

Brimonidine powder (Batch No. AG08-PK20200701) was purchased from Xingkaiyue Biotechnology (Shenzhen, China). Xylazine hydrochloride injection (2 mL/0.2 g, Batch No. 20191002) was purchased from Best Biological Technology Institute (Changsha, China). Chloral hydrate powder (Batch No. 2020304) was purchased from Kermel Chemical Reagent (Tianjin, China). Pentobarbital (Batch No. 127K1005) was purchased from Huanyu Biotechnology (Beijing, China).

Chloral hydrate was prepared as 10% chloral hydrate solution in normal saline. Pentobarbital was prepared as 3mg/mL and 4 mg/mL solutions in physiological saline. A five-fold gradient brimonidine solution series at concentrations of 0.04, 0.008, and 0.0016 mg/mL was prepared for the writhing test. A 1.2-fold gradient brimonidine solution series at concentrations of 5.5, 6.7, 8.0, and 9.6 mg/mL was prepared to determine the ED_{50} for intraperitoneal anesthesia of mice. For the Brimonidine A group, a 1.1-fold gradient solution series at concentrations of 4.5, 4.9, 5.4, and 6.0 mg/mL was used to determine the ED_{50} for intravenous anesthesia in rabbits. For the Brimonidine B group, a 1.1-fold gradient solution series at concentrations of 37.5, 41.5, 45.5, and 50.0 mg/mL was used to determine the ED_{50} for muscular anesthesia in rabbits. A 0.85-fold gradient brimonidine solution series at concentrations of 52.1,

44.2, 37.6 ,and 32.0 mg/mL was used to determine LD₅₀ by intraperitoneal injection in mice. A 0.9-fold gradient brimonidine solution series at concentrations of 18.0, 16.4, 14.9, 13.5, and 12.3 mg/mL was used to determine LD₅₀ by intravenous injection in rabbits.

To combine brimonidine and chloral hydrate, brimonidine at a dose of 2 mg/mL was added to a 10% solution of chloral hydrate. To combine brimonidine and pentobarbital, brimonidine at a dose of 1 mg/mL was added to a 3mg/mL solution of pentobarbital.

Potentialiation of pentobarbital sleeping time in mice

Mice were randomly divided into experimental, control, and threshold-dose pentobarbital groups (six mice per group, three male and three female). Each animal in the experimental group was intraperitoneally injected with a combination of brimonidine and pentobarbital at a dose of 0.1 mL/10 g, while the control group and threshold-dose pentobarbital group were intraperitoneally injected with 4 mg/mL and 3 mg/mL pentobarbital solution at a dose of 0.1 mL/10 g, respectively. Mice were then placed in an incubator to maintain their body temperature. Whether the righting response disappeared was recorded, as was the time of disappearance and recovery of the righting response. Onset of sleep was the time that animals stayed immobile and lost their righting reflex.

Assessment of analgesic activity using an acetic acid-induced writhing test

Mice were randomly divided into high-dose (0.4 mg/kg), medium-dose (0.08 mg/kg), low-dose (0.016 mg/kg), and control groups, with three males and three females per group. Mice in high-, middle-, and low-dose groups were intraperitoneally injected with 0.04, 0.008, and 0.0016 mg/mL brimonidine solution at a dose of 0.1 mL/10 g, respectively, while mice in the control group were intraperitoneally injected with normal saline at a dose of 0.1 mL/10 g. Mice in the control group were intraperitoneally injected with 0.1 mL/10 g saline. Fifteen minutes after administration of all treatments, mice were intraperitoneally injected with 0.6% acetic acid at a dose of 10 mL/kg. Numbers of writhing responses observed during a 20-min period were counted and recorded. Each writhing, defined as the stretching of the abdomen and/or stretching of at least one hind limb, was recorded with a stopwatch.

The percentage of analgesic activity was calculated as follows:

$$\% \text{ inhibition} = (W_c - W_t) / W_c \times 100\%$$

where W is the number of writhings, c is the negative control, and t is the test.

Determination of hypnotic ED₅₀ of intraperitoneal injection in mice

Mice were divided into four groups (five males and five females per group) that were administered 55.6, 66.7, 80.0, or 96.0 mg/kg brimonidine. Mice in each group were intraperitoneally injected with a 1.2-fold

gradient brimonidine solution series at a dose of 0.1 mL/10 g. When mice were still in the supine position, they were placed on the heat preservation table in the supine position to maintain their body temperature.

Observation indicators: (1) Behavioral and autonomous activities of mice: the appearance, posture, and mental state of mice were observed as activity (lifting and walking) and inactivity (prostrate without motion). (2) Ratio of sleeping: the incidence of loss of righting response was observed following drug administration. The index of sleep falling was evaluated as the loss of righting response for more than 1 min. Numbers for each group were observed in mice sleeping within 1 h. (3) Sleeping time: the time from administration to the disappearance of righting response and recovery of righting response was recorded. The time from drug administration to disappearance of the righting response was considered the sleep latency, while the time from disappearance of the righting response to recovery of the righting response was considered the sleep duration.

Calculation of ED_{50} by modified Karber method

$$ED_{50} = \lg^{-1}[X_m - I(\Sigma P - 0.5)]$$

X_m : logarithmic value of the maximum-dose group dose

I : Logarithmic ratio of high-dose to low-dose for two adjacent groups

P : Ratio of sleeping of animals in each group, expressed as a decimal

$$ED_{50} 95\% CI = \lg^{-1}(\lg ED_{50} \pm 1.96 S_{\lg ED_{50}})$$

$$S_{\lg ED_{50}} = I * [(\Sigma P - \Sigma P^2) / (n - 1)]^{0.5}$$

N : number of animals in each group

Determination of hypnotic ED_{50} of intravenous rabbits

Rabbits were divided into four groups ($n = 10$): 4.5 mg/kg, 4.9 mg/kg, 5.4 mg/kg, and 6.0 mg/kg. Rabbits in each group were injected with a 1.1-fold gradient solution series of Brimonidine A at a dose of 1.0 mL/kg. When rabbits were inactive, they were required to assume the supine position.

Observation indexes: (1) Behavioral and autonomic activities of rabbits: the appearance, posture, and mental state of rabbits were characterized by when they walked with or without using their legs (considered as activity), while their prostration was considered as quiet. (2) Ratio of sleeping: the incidence of disappearance of acupuncture response after medication was observed. The index of acupuncture sleep falling was calculated as the disappearance of acupuncture response for more than 1 minute, while sleep falling numbers were observed for 1 h in each group. (3) Sleeping time: time from the disappearance of acupuncture response to the recovery of acupuncture response was calculated as the sleeping time.

The modified Karber method was used to calculate ED_{50} , as described above.

Determination of hypnotic ED_{50} of rabbits injected intramuscularly

Rabbits were divided into four groups ($n = 10$): 7.5 mg/kg, 8.3 mg/kg, 9.1mg/kg, and 10.0 mg/kg. Rabbits in each group were injected with a 1.1-fold gradient solution series of Brimonidine B at a dose of 0.2 mL/kg. When rabbits were still in the supine position, they were required to assume the supine position.

Observation indexes and the calculation method of ED_{50} were identical to those described above for the determination of hypnotic ED_{50} of intravenous injection hypnosis in rabbits.

Determination of hypnotic ED_{50} of rabbits injected intrarectally

Rabbits were divided into four groups ($n = 10$): 7.5 mg/kg, 8.3 mg/kg, 9.1mg/kg, and 10.0 mg/kg. Rabbits in each group were injected with a 1.1-fold gradient solution series of Brimonidine B at a dose of 0.2 mL/kg.

Observation indexes and the calculation method of ED_{50} were identical to those described above for the determination of hypnotic ED_{50} of intravenous and intrarectal injection hypnosis in rabbits.

Measurement of LD_{50} and therapeutic index of mice after intraperitoneal injection using the up-down method

Seventeen KM mice were intraperitoneally injected with a 0.85-fold gradient brimonidine solution series at a dose of 0.1 mL/10 g, one at a time. After the mice rested, they were placed in an incubator to maintain their body temperature.

If the first animal survived, the second animal was given a higher dose. However, if the first animal died or was dying, the second animal was administered a lower dose to obtain a total number of experiments n and the mortality p of each dose.

Calculation of LD_{50} :

$$LD_{50} = \lg^{-1}[\Sigma(nx)/\Sigma n]$$

$S_{\lg LD_{50}} = d \cdot \{\Sigma[p(1-p)/(n-1)]\}^{0.5}$, where d is the log difference between two adjacent doses.

$$LD_{50} \text{ 95\% CI} = \lg^{-1}(\lg LD_{50} \pm 1.96 S_{\lg LD_{50}})$$

$$\text{Therapeutic index} = LD_{50}/ED_{50}$$

Measurement of LD₅₀ and therapeutic index of rabbits after intravenous injection using the up-down method

Rabbits were intravenously injected with a 0.9-fold gradient brimonidine solution series at a dose of 10 mL/1kg, one at a time. LD₅₀ was measured and calculated as described above for the determination of LD₅₀ in mice.

Synergistic effects of brimonidine and chloral hydrate on hypnosis in rabbits

Twenty-four rabbits were divided into three groups (eight rabbits per group): low-dose, high-dose, and control groups. The low-dose group was intravenously injected with 0.3 mL/kg brimonidine and chloral hydrate, the high-dose group was injected with 0.6 mL/kg brimonidine and chloral hydrate. After intramuscular injection of 0.15 mL/kg xylazine, the control group was injected with 1.5 mL/kg 10% chloral hydrate at a rate of 2–3 mL/min.

Behavioral and voluntary activities were recorded from the time of administration to the disappearance and recovery of acupuncture responses. Sleep latency was recorded as the time between pentobarbital administration and sleep onset. Sleeping time was observed as described above for the determination of hypnotic ED₅₀ in rabbits given intravenous injection. After the acupuncture response recovered, rabbits in low-dose and high-dose groups were intravenously injected with 0.15 mL/kg brimonidine and chloral hydrate, and then the re-sleeping time was observed.

Statistical test

One-way ANOVA and Dunnett's t-test were used to calculate the significance of potentiation of pentobarbital sleeping time and writhing tests in mice, as well as the synergistic effect of brimonidine and chloral hydrate. Statistical analysis was performed by SPSS 17.0 (IBM, Armonk, NY, USA).

Results

Brimonidine potentiates pentobarbital hypnosis

After injection, mice in the experimental groups gradually developed ataxia and decreased exercise. Approximately 3–5 min after injection, mice exhibited a negative rollover response and the ratio of sleeping was 100%, as defined by an increased sleeping time (118.7 ± 4.6 min) in treated mice compared with control groups (14.8 ± 4.4 min, Figure 1). Most animals urinated within 0.5 h of administration.

After intraperitoneal injection of pentobarbital in the threshold-dose (30 mg/kg) and control groups (40 mg/kg), mice developed ataxia, decreased their mobility, and then lay down quietly. However, during the 2-h observation period, the ratio of sleeping in the threshold-dose pentobarbital group was always 0, and no micturition occurred.

Writhing test in mice

Following intraperitoneal injection of brimonidine for 15 min, high-dose group mice exhibited decreased activity. The middle-dose group was still active, but there was no courtship action, whereas the low-dose group moved freely and exhibited courtship actions such as raising forelimbs. In the high-dose group (0.4 mg/kg), there was no writhing response within 20 min of intraperitoneal injection of acetic acid solution. In the middle-dose group (0.08 mg/kg), most mice showed writhing reactions.

In the acetic acid-induced writhing model, the low-dose group exhibited a significant analgesic effect with 82.5% reduction in writhing responses at a dose of 0.016 mg/kg compared with the control group (Table 1). Brimonidine showed a good analgesic effect that was enhanced further at higher doses.

Table 1
Analgesic activity of brimonidine assessed by acetic acid-induced writhing in mice.

Group	Dose mg/kg	Number of writhes	% Inhibition
Control	0	63.0 ± 7.3	-
High dose	0.4	0***	100
Medium dose	0.08	7.8 ± 4.3***	87.6
Low dose	0.016	10.6 ± 4.5***	82.5
*** Compared with the control group, p < 0.001 (one-way ANOVA, followed by Dunnett's test).			

Hypnotic ED₅₀ of intraperitoneal injection in mice

After injection, mice first exhibited anxiety, followed by ataxia, decreased exercise, slow and deepened breathing, white fundus, and obvious micturition. Moreover, some mice exhibited negative, positive, and negative responses one after another. The ratio of sleeping in the 96 mg/kg group reached 100% (Table 2), but the sleeping time was only 4.8 ± 0.5 min.

Table 2
Hypnotic effects of intraperitoneal brimonidine injection in mice

Group	Dose mg/kg	N	Hypnotic number	Ratio of sleeping %
1	55.6	10	0	0
2	66.7	10	2	20
3	80.0	10	6	60
4	96.0	10	10	100

Hypnotic effects were summarized in Table 3. The hypnotic ED₅₀ of brimonidine solution was 75.7 mg/kg, and the 95% confidence interval (95% CI) was 70.2–81.6mg/kg.

Hypnotic ED₅₀ of intravenous injection in rabbits.

During half injection, rabbits struggled impatiently for several seconds and then calmed down. During the incubation period, the front legs were straight, the body was shaking, the tail was straight, the pupils became narrowed, the fundus was pale, and the limbs gradually relaxed. During sleep, the rabbit may experience phenomena such as eyeball shaking, micturition, and body surface temperature drop, or may wake up temporarily by changing the body position or twisting the limbs.

Table 3
Hypnotic effects of intravenous brimonidine in rabbits

Group	Dose mg/kg	N	Hypnotic number	Ratio of sleeping%
1	4.5	10	0	0
2	4.9	10	3	30
3	5.4	10	7	70
4	6.0	10	10	100

Hypnotic effects were summarized in Table 3. The hypnotic ED₅₀ of brimonidine solution was 5.2 mg/kg and the 95% CI was 5.0–5.4 mg/kg.

In the 6-mg/kg dose group, all rabbits entered the sleep stage, the induction period was 8.2 ± 3.3 min, and sleep duration was 128.1 ± 16.9 min (Figure 2). However, during the induction period, two rabbits exhibited obvious extrapyramidal reactions (e.g., arch inversion, head and neck elevation, muscle tremor) and

sleeping times of only 6 and 9 min, which were significantly lower than other rabbits in the same group. To verify whether the sleeping time of these two rabbits was shortened due to over-anesthesia, 4.9 mg/kg brimonidine solution was intravenously injected into rabbits three days later. Subsequently, no obvious extrapyramidal reaction occurred during the induction period and sleeping times were prolonged to 46 min and 90 min, respectively.

Hypnotic ED₅₀ of intramuscular injection in rabbits

There was no restlessness or struggle during intramuscular injection of rabbits. Moreover, the latency response was obviously reduced compared with intravenous injection, the sleep induction period was less than 3 min, and most rabbits did not exhibit muscle tremor or horn arch rebound. During sleep, rabbits exhibited symptoms such as nystagmus, micturition, and temperature drop on the body surface. Changing the body position or twisting the limbs could temporarily wake up the rabbits.

Table 4
Hypnotic effects of intramuscular brimonidine in rabbits

Group	Dose mg/kg	N	Hypnotic number	Ratio of sleeping %
1	7.5	10	0	0
2	8.3	10	2	20
3	9.1	10	7	70
4	10.0	10	10	100

Hypnotic effects were were summarized in Table 4.The hypnotic ED₅₀ of intramuscular brimonidine injection was 8.8 mg/kg and the 95% CI was 8.5–9.1 mg/kg.

All rabbits in the 10-mg/kg group fell asleep, with an induction period of 6.4 ± 1.8 min and sleeping time of 19.0 ± 11.3 min(Fiture 2).

Hypnotic ED₅₀ of intrarectal injection in rabbits

There was no restlessness or struggle during intrarectal injection of rabbits. The muscle tremor or horn arch rebound was not observed.

Table 5
Hypnotic effects of intrarectal brimonidine in rabbits

Group	Dose mg/kg	N	Hypnotic number	Ratio of sleeping %
1	7.5	10	1	10
2	8.3	10	3	30
3	9.1	10	6	60
4	10.0	10	10	100

Hypnotic effects were summarized in Table 5. The hypnotic ED₅₀ of intrarectal brimonidine injection was 8.7 mg/kg and the 95% CI was 8.3–9.1 mg/kg.

All rabbits in the 10-mg/kg group fell asleep, with an induction period of 11.0 ± 4.1 min and sleeping time of 53.6 ± 27.9 min (Figure 2).

LD₅₀ of mice was determined with an up-and-down sequence

Survival and death of mice after intraperitoneal brimonidine injection with up-and-down sequence were summarized in Table 6.

Table 6 Survival and death of mice after intraperitoneal brimonidine injection

Dosage mg/kg	Logarithmic dose	Animal number																
		1	2	2	4	5	6	7	8	9	10	11	12	13	14	15	16	17
521	2.72							○		○								
442	2.65						□		□		○		○		○			
376	2.58	○		○		□						□		○		○		○
320	2.51		□		□												□	

○ Mouse died after injection, □ Mouse survived after injection

The LD₅₀ of intraperitoneal brimonidine injection in mice was calculated to be 379 mg/kg, with a 95% CI of 343–420 mg/kg and therapeutic index of 5.0.

LD₅₀ of mice was determined with an up-and-down sequence

Survival and death of rabbits after intraperitoneal brimonidine injection were summarized in Table 7.

Table 7 Survival and death of rabbits after intravenous brimonidine injection

Dosage mg/kg	Logarithmic dose	Animal number																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
180	2.25								○										
164	2.21							□		○		○							
149	2.17				○						□				○				
135	2.13	○		□		□							○				○		
123	2.09		□											□		□		○	□

○ Rabbit died after injection, □ Rabbit survived after injection

The LD₅₀ of intravenous brimonidine injection in rabbits was calculated to be 146 mg/kg, with a 95% CI of 135-157mg/kg and therapeutic index of 28.0.

Synergistic effects of brimonidine and chloral hydrate

The induction periods of low-dose and high-dose groups were shorter than 1 min. After intravenous injection, all animals exhibited miosis, white fundus, turning over, decreased muscle tension, and no limb stiffness. Rabbits in both groups entered the anesthesia period with similar sleeping times. During the anesthesia period, rabbits exhibited nystagmus and decreased body surface temperatures, but did not urinate and could not temporarily wake up by changing the body position or twisting the limbs. In the control group, the induction period was 3.3 ± 1.4 min, mild symptoms (such as limb stiffness) were exhibited, and one death occurred. The sleeping time in control group was shorter than observed in high dose group (Figure 3; $P < 0.01$). There was no difference in sleeping times between low-dose and high-dose groups (Figure 3; $P > 0.05$).

Discussion

Brimonidine is clinically used in the treatment of glaucoma and facial congestion. No previous study has investigated the induction or maintenance of general anesthesia with brimonidine; however, a report of brimonidine administration before general anesthesia in animals, such as intraperitoneal injection of 30µg/kg brimonidine, diminished the sympathetic nerve effect of ketamine general anesthesia in rats [1]. In addition, there are many reports regarding the use of brimonidine for local anesthesia studies. For example, preoperative use of brimonidine eye drops to prevent an increase in intraocular pressure also improved the local analgesic effects of ophthalmic operations, such as cataract phacoemulsification [2]. Moreover, 0.33% brimonidine gel combined with lidocaine cream prolonged the local anesthesia time in skin surgery [3]. Inhibition of thermal nociception in mice following epidural administration of brimonidine had an analgesic ED₅₀ of 0.37 nM, and the combination of brimonidine with an epidural, such as opioids,

can lead to synergistic analgesia ^[4]. Epidural administration of brimonidine also had analgesic effects in a rat model of chronic neuropathic pain ^[5, 6].

Many clinical reports indicate that local and systemic administration of brimonidine can cause side effects in the central nervous system, resulting in nervous system symptoms such as sedation, lethargy, and even death ^[7]. Following intravenous injection of 1 mg/kg brimonidine, the rats stopped moving, their pupils dilated (different from those of primates and rabbits, which may be related to species specificity), and they calmed down. After intravenous injection of 2 mg/kg brimonidine, the rats breathed slowly and quietly, and the dose did not reach the level of subliminal anesthesia ^[8]. Toxicity testing revealed that a large dose (5 mg/kg) of oral brimonidine elicited short-term hypnotic and sedative effects in rabbits, which also exhibited disappearance of nerve responses and reduced muscle tension ^[9]; however, the effect of general anesthesia was not studied in depth.

This study confirmed that brimonidine has good hypnotic, sedative, and analgesic effects on mice and rabbits. This result is related to the α_2 receptor agonist action of brimonidine.

Human and mammalian α_2 -adrenergic receptors belong to a family of seven-transmembrane G protein-coupled receptors, which can transfer G_i/G_o protein and have the following effects: inhibition of adenylate cyclase and protein kinase A; activation of protein kinase C; activation of potassium channels to hyperpolarize cells and produce postsynaptic inhibition; and inhibition of calcium influx and neurotransmitter release at nerve endings, leading to presynaptic inhibition ^[10]. There are three α_2 receptor subtypes in humans, primates, pig, and rabbit (α_{2a} , α_{2b} , and α_{2c}), which produce different effects. The K_i of human α_{2a} , α_{2b} , and α_{2c} receptors for brimonidine are 3.7, 512, and 120 nM, respectively ^[11].

The locus coeruleus brainstem is an important functional area in the central nervous system that regulates arousal. Activation of α_{2a} receptors hyperpolarizes nerve cells in the locus coeruleus, which reduces the release of norepinephrine and inhibition of γ -GABA neurons in the ventrolateral anterior cross nucleus of the hypothalamus to induce sedative hypnotic effects. The ED_{50} of brimonidine for inducing potassium influx in rat locus coeruleus neurons *in vitro* is 4.6×10^{-8} M, while 10^{-6} M brimonidine can induce the maximum current ^[12]. The hypnotic mechanism of brimonidine is similar to that observed in normal non-eye-moving sleep. During observations of the hypnosis of rabbits, we found that the eyeball moves back and forth rapidly and slightly, and it is speculated that the rabbits entered a state similar to sleep.

The hypnotic assessment method was based on prolongation of sleep induced by pentobarbital, a classic pharmacological method for screening sedative hypnotic agents ^[13, 14]. The hypnotic effect was evaluated by sleep time duration induced by a subhypnotic dose of pentobarbitone. Brimonidine showed hypnotic activity that increased sleeping time induced by pentobarbitone.

In this study, we found that the ED₅₀ of mouse hypnosis (78 mg/kg, intraperitoneal administration) and rabbit hypnosis (5.20 mg/kg, intravenous injection; 8.8 mg/kg, intramuscular injection; 8.7 mg/kg, intrarectal injection), and sleeping time of mice (4.8 ± 0.5 min, intraperitoneal injection) and rabbits (128.1 ± 16.9 min, intravenous injection; 19.0 ± 11.3 min, intramuscular injection; 53.6 ± 27.9 min, intrarectal injection). The observed difference in hypnotic doses of brimonidine in mice and rabbits may be related to the difference in species. Thus, if brimonidine is developed as an anesthetic for experimental animals, it is more suitable to be used alone in rabbits than mice.

The α₂ receptor agonist can exert analgesic and noxious stimulation inhibitory effects in peripheral nerves, spinal cord, and upper spinal cord [15]. The α_{2a} subtype is contained in the spinal dorsal horn and primary sensory neurons, whereby adrenergic receptor agonists bind this receptor to trigger postsynaptic inhibition of secondary afferent neurons and presynaptic inhibition of primary sensory neurons, thus playing a major role in inhibiting pain signals [16]. The spread of chronic inflammation and neuropathic pain can also be inhibited by the anti-sympathetic nerve pathway. Activation of the α_{2c} receptor inhibits the activity of nociceptive neurons in the dorsal root ganglion and spinal dorsal horn, but also reduces the release of endogenous opioids, leading to complex analgesic effects [17].

The acetic acid-induced writhing reflex mouse model is a widely accepted, simple, sensitive, and effective pain model for evaluating peripherally acting analgesics. The pain caused by acetic acid liberates endogenous substance to elicit peripheral- and central-mediated analgesic actions [18]. In the writhing test of mice, the analgesic effect of brimonidine at a dose of 0.016 mg/kg was obvious—this dose is 4,800 times lower than the hypnotic ED₅₀. Compared with 0.016 mg/kg brimonidine, 0.08 mg/kg brimonidine had a sedative effect and an increased analgesic effect. A dose of 0.4 mg/kg brimonidine decreased the activity of mice, and no acetic acid-induced peritoneal pain reaction was observed. These results indicate that brimonidine significantly reduced the number of writhes in a dose-dependent manner compared with the negative control.

The hypnotic effect of brimonidine was more obvious in rabbits than in mice. Our experiments found that intravenous administration of 4.5 mg/mL brimonidine could make the righting response disappear. During this period, the limbs could be placed at will, but pinching the limbs could cause muscles of the limbs to contract. Although α₂ receptor agonists can theoretically cause presynaptic inhibition of motor neurons and play a certain role in muscle relaxation [19], muscle relaxation is mainly caused by higher central inhibition. The limb muscle contraction caused by the clamp proves that the sedative effect of the above dose was good, but it did not achieve a hypnotic effect. The subthreshold dose of brimonidine in rabbits was far less than the anesthetic effective dose (not obvious in mice), but a good sedative effect at the low dose still caused some interference in hypnotic dose experiments in rabbits. Therefore, in the hypnotic half-effective-amount experiment of rabbits under intravenous intramuscular anesthesia, the acupuncture response rather than the righting response was specifically selected as the test criterion for sleep. The dose of brimonidine that inhibited the acupuncture response maintained the depth of

anesthesia for simple surgery in small animals, while the low dose of brimonidine was suitable for pre-physical sedation.

Regarding the hypnotic effect of brimonidine on rabbits, pupil contraction was observed and the time of pupil contraction was very similar to the time of sedative action. The degree of pupil contraction can be used to roughly evaluate the agonistic performance of highly selective α_2 receptor agonists on central α_2 receptors of rabbits, monkeys, and other animals [20]. α_2 receptor agonists stimulate α_2 receptors in the postsynaptic membrane of neurons in the oculomotor nucleus, thereby inhibiting the release of noradrenaline from sympathetic nerve endings. This effect makes the contraction of pupil sphincter more dominant than the contraction of pupil opening muscle, resulting in pupil contraction. Brimonidine is insensitive to α_1 receptors. When the local concentration reaches 10^{-4} M, contractile force of the psoas major muscle is only 8%, which is not enough to offset central mydriasis [19]. It was also found that the conjunctiva of rabbits and mice turned white after administration of brimonidine, because brimonidine caused the contraction of anterior small blood vessels by activating α_{2a} receptors of small blood vessels; however, brimonidine did not easily cause contraction of retinal blood vessels [21]. A local brimonidine concentration of 10^{-4} M had no significant influence on retinal blood supply or function [22].

Although brimonidine has hypnotic and analgesic effects because it indirectly inhibits the ascending activation system of the reticular structure through the locus coeruleus, it does not block the awakening mechanism while accompanying sleep [23]. We found that during the hypnotic process elicited by injected administration of brimonidine to rabbits, they would be awakened in a short time by changing their body position and pulling their limbs hard. Therefore, brimonidine alone is only suitable for minor operations that do not involve frequent physical renewal. In addition, animals urinated during anesthesia, which might be due to the high dose of brimonidine acting on α_2 receptors in the paraventricular nucleus of the hypothalamus, thereby inhibiting the release of antidiuretic hormone to cause an increase in urine output. Simultaneously, brimonidine inhibited norepinephrine release and contraction of the bladder sphincter, leading animals to urinate. Dexmedetomidine had a similar effect on diuresis in clinical applications [24]. This is disadvantageous for simple anesthesia of animals without catheter insertion because sudden micturition can affect disinfection of the surgical area.

After a high dose of brimonidine was intravenously administered, the hypnotic effect was not enhanced, but weakened. During the induction of general anesthesia, high-dose α_2 receptor agonists might activate dopamine neurons in the ventral tegmental area and increase dopamine concentrations in the related forebrain projection areas [25]. During anesthesia, extrapyramidal symptoms appeared in some rabbits, and the extrapyramidal reaction might further weaken the inhibitory effect of drugs on central adrenal energy and affect the anesthetic effect.

To overcome these shortcomings and solve potential problems with future application, the combination of brimonidine with other anesthetics is a good method. In clinical anesthesia, dexmedetomidine can strengthen the efficacy of other central sedative drugs and analgesic drugs, and reduce the dosage of

other drugs, thus reducing toxic and side effects. The present study found that brimonidine, a highly selective α_2 receptor agonist with an imidazoline ring, has a similar effect. The results of experiments examining the hypnotic effect of brimonidine on increasing the threshold dose of pentobarbital confirmed the hypnotic effect of brimonidine. Moreover, these results revealed that 10 mg/kg brimonidine combined with 30 mg/kg pentobarbital hypnotized all mice for more than 2 h after intraperitoneal injection, without any death phenomenon, which is beneficial for long-term operations of mice. However, α_2 receptor agonists such as brimonidine can lead to hypothermia [26], meaning small animals such as mice and rats may be injured or die. Therefore, in this study, mice were incubated to maintain a normal physiological body temperature.

In addition to pentobarbital, a combination of brimonidine and chloral hydrate was also found to have a good synergistic effect. When brimonidine and chloral hydrate were used together, the doses of the two drugs in the low-dose group were only one-tenth of the original single dose. In addition, the low-dose group exhibited rapid onset of anesthesia, a stable anesthesia effect, and convenient supplement of anesthetics. Although the dosage of the high-dose group was double that of the low-dose group, the anesthetic effect was similar. Thus, combining brimonidine and chloral hydrate has the potential advantages of stable effects and high cost performance for animal anesthesia.

Chloral hydrate, a central inhibitor, is seldom used alone at present. Mainly used for sedation and auxiliary anesthesia, chloral hydrate can quickly induce drowsiness and sedation, and has the advantages of fast anesthesia induction and recovery, high cost performance, and reliable sources. However, due to its limited analgesic effect, the dose required for anesthesia is close to the toxic amount and the safety range is small. Excessive chloral hydrate can inhibit myocardial contraction of animals and shorten the refractory period, leading to respiratory depression and death [27].

Chloral hydrate obviously accelerated the induction time of brimonidine anesthesia, and the application of brimonidine overcame the disadvantage of unsatisfactory anesthesia depth and muscle relaxation effects of chloral hydrate alone. Indeed, the reduced mixture had good anesthesia depth and muscle relaxation effects. The synergistic mechanism involves chloral hydrate further enhancing the analgesic effect of α_2 receptor agonists by inhibiting the synaptic function of γ -aminobutyric acid (GABA) neurotransmission, whereas the inhibitory effects of the two drugs on respiratory and cardiovascular diseases may not exhibit synergy [28]. In addition, the combined use of drugs also reduces their respective doses, which is beneficial for both the effectiveness and safety of combined anesthesia.

The synergistic effect of brimonidine and chloral hydrate was compared with the previously reported formula of xylazine and chloral hydrate [29]. Xylazine, a non-imidazoline cyclic middle-selective α_2 receptor agonist only used in veterinary medicine, is cheap and can be used for animal anesthesia with sedative drugs such as chloral hydrate. After intramuscular injection of xylazine alone, the anesthetic effect is uncertain and additional anesthesia is prone to accidents. Spasm and tremor are obvious during the induced excitement period; the maintenance period of anesthesia is short; and the decrease of muscle

tension is often not significant. Moreover, xylazine readily influences cardiovascular functions and causes arrhythmia.

Experiments revealed that the individual effects of combined anesthesia with xylazine and chloral hydrate varied significantly, and one animal died after anesthesia, which was related to the limited drug consumption and high self-risk of xylazine and chloral hydrate in combination. In addition, a previous report indicated that chloral hydrate combined with xylazole, another α_2 receptor agonist with low receptor selectivity, can cause obvious arrhythmia in anesthetized animals ^[30]. In contrast, brimonidine has higher receptor selectivity and lower cardiovascular side effects than low-selectivity α_2 receptor agonists ^[31].

Two rabbits in the chloral hydrate combined with xylazine group were found to have ear vein inflammation a few days later, which may have been caused by the chloral hydrate solution. No ear vein inflammation was observed in high-dose or low-dose groups administered chloral hydrate combined with brimonidine, which may be related to the low dosage of the combined medicine.

In terms of safety, the therapeutic index of intraperitoneal brimonidine administration in mice was 5.0, while the therapeutic index of intravenous brimonidine in rabbits was 28.0, indicating that the safety range of brimonidine was wider in rabbits than mice. In this study, brimonidine was administered in combination with pentobarbital and chloral hydrate, and no deaths occurred.

There have been many reports on the safety of brimonidine administered to animals at high doses; for example, oral administration of brimonidine to rats for up to 1 year (2.5 mg/kg/day) did not cause organ toxicity ^[8]. In a study of reproductive toxicity, oral administration of 5 mg/kg brimonidine to pregnant rabbits caused abortion in individual rabbits, and had no teratogenic effect on offspring ^[9]. According to pharmacokinetic studies ^[32], brimonidine is metabolized in the liver and excreted in urine, with a half-life for blood elimination of 3 h and 29% binding rate of plasma protein. Such experimental conclusions have reference value for the use of brimonidine.

This sleeping time of rabbits in the 10-mg/kg intramuscular anesthesia group was shorter than observed in the 10-mg/kg intrarectal anesthesia group (Figure 2; $P < 0.05$). Use of brimonidine in clinical eye drops as a drug for glaucoma yielded high local absorption and no local tissue irritation ^[32] because it has proper physical properties ($\text{LogP} = 0.83$, $\text{PKa} = 7.78$), which makes brimonidine a potential sedative and anesthetic for mucosal administration, when dexmedetomidine showed low bioavailability.

In future research, the safety of brimonidine for general anesthesia should be further investigated, such as monitoring of vital signs during anesthesia, investigating possible impacts on blood biochemistry and other indicators, and further confirming the efficacy of brimonidine combined with other anesthetics.

Conclusions

In summary, brimonidine has good sedative and analgesic effects, and is stable and safe; thus, brimonidine has the potential for use as a new general anesthesia drug with multiple routes of administration. The combined anesthesia method of brimonidine and chloral hydrate has the advantages of convenient operation, short induction time, long maintenance time, good analgesic and muscle relaxation effects, low anesthetic dosage, low anesthetic adverse reaction, stable anesthetic effects, high cost performance, and reliable sources. The use of brimonidine alone or in combination with other anesthetics may be a simple, rapid, safe, and effective method to induce sedation and anesthesia in animal experiments and even clinical surgery.

Declarations

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Author Contributions:

Conceptualization, C.B., W.X.H., S.M., Z.T. and L.X.; Methodology, C.B. and W.X.H.; Validation, C.B., W.X.H., S.M. and L.X.; Formal Analysis, C.B., W.X.H., S.M., G.P., Z.T. and L.X.; Investigation, C.B., W.X.H., G.P. and Z.T.; Resources, C.B., W.X.H., S.M., G.P. and L.X.; Data Curation, C.B., W.X.H. and Z.T.; Writing, C.B. and W.X.H.; Funding acquisition, G.P.; Supervision, G.P. and Z.T.

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The authors declare no conflict of interest.

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Figures

Figure 1

The sleeping time of mice with intraperitoneal injection. Data shown as mean $\pm$ SEM.*** P < 0.001 (one-way ANOVA, followed by Dunnett's test).

Figure 2

The Sleeping time of rabbits with intravenous (6.0mg/kg) , intramuscular (10.0mg/kg)and intrarectal (10.0mg/kg) brimonidine injection.Data shown as mean $\pm$ SEM.* $P < 0.05$; *** $P < 0.001$ (one-way ANOVA, followed by Dunnett's test)

Figure 3

The Sleeping time of Control group (0.15mg/kg Xylazine + 0.15mg/kg Chloral hydrate),Low dose group(0.6mg/kg Brimonidine+0.03mg/kgChloral hydrate) and High dose group (1.2mg/kg Brimonidine+0.06mg/kgChloral hydrate) ** $P < 0.01$ (one-way ANOVA, followed by Dunnett's test)

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