Anesthesia using three kinds of mixed anesthetics (mouse)

Target audience: Those who have knowledge about anesthetics

Target animals: Mainly mice

Research Institute, National Center for Global Health and Medicine

Tadashi Okamura

Investigation of anesthetic effects of three kinds of mixed anesthetics

strain (sex)	age in weeks	body weight (g)	Righting reflex disappearance time(min)	Surgical anesthesia start time(min)	Surgical Anesthesia endtime (min)	Surgical anesthesia time
M/M/8: 0.3/4/5						
C578U/6NCr(male)	12	25.3 ± 1.2	3.6 ± 1.3	5.4 ± 1.1	67.6 ± 2 .5	62.3 ± 30.2
C578U/6NCr( )	10	20.7 ± 1.1	3.2 ± 1.0	5.0 ± 0.5	11 .0 ± 7.5	114.0 ± .
ICR(male)	11	44.2 ± 1.1	3.5 ± 2.0	7.3 ± 3.5	62.3 ± 14.7	55.0 ± 11.
ICR( )	10	35.4 ± 1.0	3.0 ± 0.4	6.6 ± 0.5	5.1 ± 4.5	.5 ± 4.1

P<0.05, *P*<0.05, +*P*<0.01,++*P*<0.01compared with the same strain. Mean ± SD

Anesthetic effect of triple anesthetics stored at room temperature

Elapsed time after anesthesia adjustment	Righting reflex disappearance time(mn)	Surgical anesthesia start time(mn)	Surgical anesthesia end time(mn)	surgical anesthesia time (mn)
M/M/8: 0.3/4/5				
1st day	3.6 ± 1.3	5.4 ± 1.1	67.6 ± 29.5	62.3 ± 30.2
4 weeks	4.3 ± 0.3	6.8 ± 0.7	77.0 ± 2.9	70.3 ± 2.3
8 weeks	4.1 ± 0.3	6.2 ± 1.0	62.8 ± 4.6	56.6 ± 5.4

C578U/6NCr, male, n=4

Purpose

In this study, we investigated the effects of a three-kind mixed anesthetic, which is attracting attention as a new injection anesthetic, for fertilized egg transplantation surgery, which is the most widely used reproductive engineering technology, on the results of mouse embryo transplantation.

Method 1: About triple anesthetics (M/M/B)

It is a mixed anesthetic containing medetomidine, an α2-adrenergic receptor agonist, midazolam, a GABAA receptor agonist, and butorphanol, an opioid (Kawai S. et al., Exp.Anim., 2011). Administration of atipamezole, which is a medetomidine antagonist, enables prompt awakening. Based on the reports by Kawai S et al. and Tohoku University Animal Research Institute, we increased the dose of medetomidine by 2.5 times (Table 2) and adjusted the dose to 0.1 ml per 10 g of body weight.

The protocol can be downloaded from http://www.rincgm.jp/department/lab/08.

Component	Product name	Distributor	Concentration	Required amount
Medetomidine	Domitor	Nippon Zenyaku Kogyo Co., Ltd.	1 mg/ml	0.75 mg/kg
Midazolam	Midazolam "Sand"	Sando Co., Ltd.	5 mg/ml	4 mg/kg
Butorphanol	Bettlefar	Meiji Seika Pharma Co., Ltd.	5 mg/ml	5 mg/kg
Atipamezole	Anti-sedan	Nippon Zenyaku Kogyo Co., Ltd.	5 mg/ml	0.75 mg/kg

Supplementary material

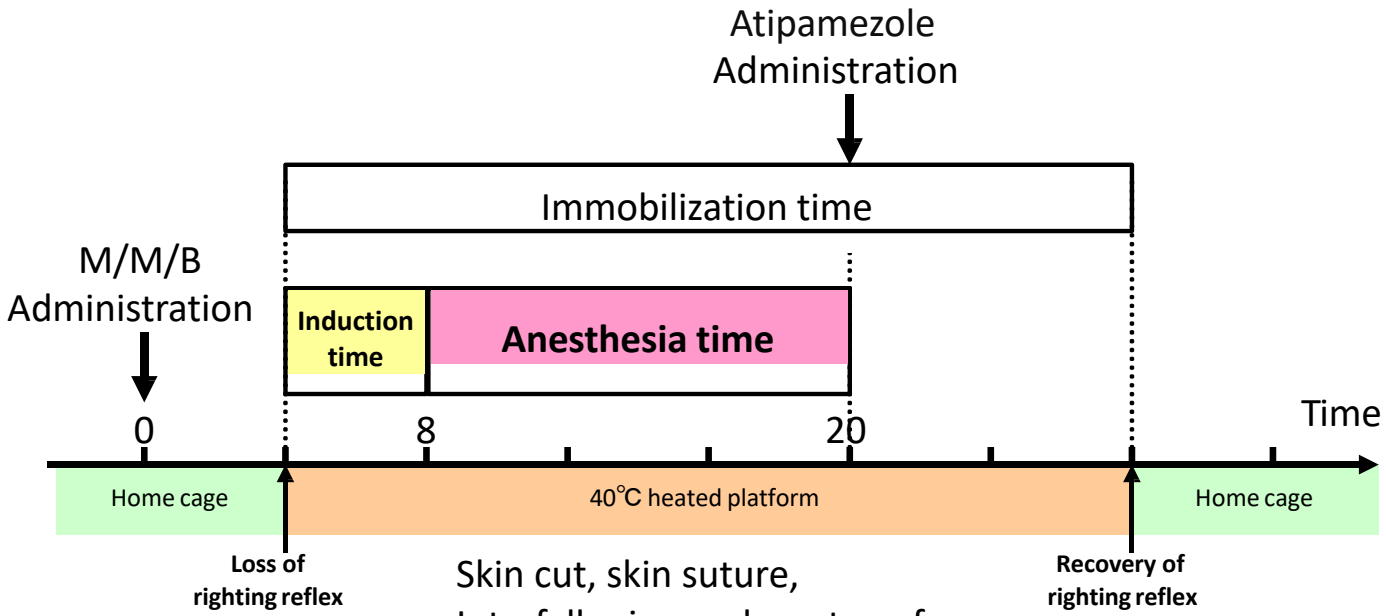
[Anesthetics and analgesics]

- Medetomidine (Domitor, Nippon Zenyaku Kogyo Co., Ltd.)
- Midazolam (Midazolam, Sandoz Co., Ltd.)
- Butorphanol (Betorfal, Meiji Seika Pharma Co., Ltd.)

Preliminary study of medetomidine dosage for mixed anesthetics

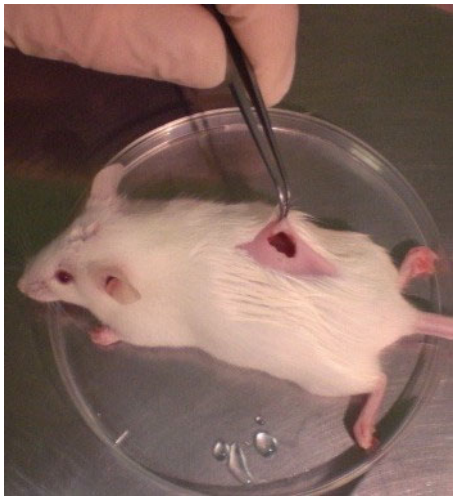
M/M/B (mg/kg)	Strain (female male)	Weight (g)	Administration site	Loss of reflex population
0.3/4/5	ICR(F)	28.6±0.5	ip	0/2
0.3/4/5	ICR(F)	28.4±0.4	sc	6/8
0.375/4/5	ICR(F)	-	ip	0/2
0.45/4/5	ICR(F)	-	ip	2/4
0.6/4/5	ICR(F)	-	ip	6/10
0.75/4/5	ICR(F)	31.1±0.3	ip	13/13
0.75/4/5	ICR(F)	28.3±0.4	sc	6/6

Time course of mouse fertilized egg transfer

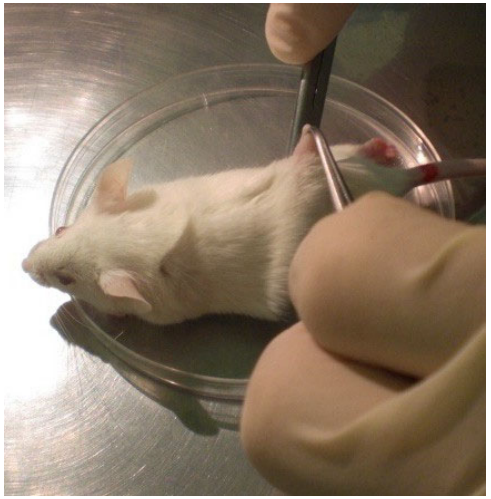


Skin cut, skin suture,
Intrafallopian embryo transfer,
Belly peeling,
Hair clipping,

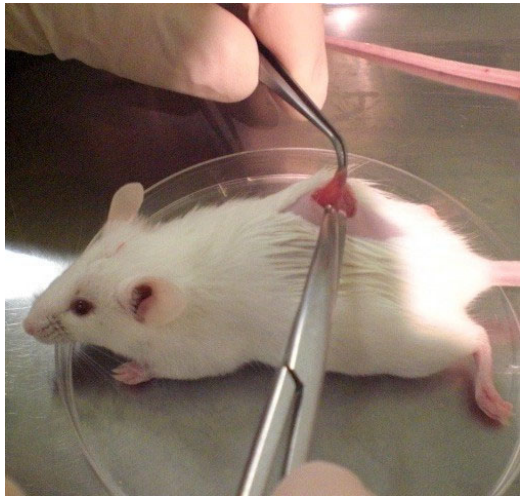
Surgery time is about 10 min/animal



1 cm skin incision



Subcutaneous dissection and approximately 1cm incision on the abdominal wall (2 locations)



Exposing ovaries and fallopian tubes

result

Production of pups following embryo transfer.

Anesthetic agent	No.of 2−cell embryos transferred*	No.of recipients	% of Implants	% of pups
0.75M/M/B	166	10	71.3±14.4	44.6±14.7
Isoflurane	182	10	68.8±20.2	42.3±13.8

*Two-cell embryos from C57BU/6NCr

There is no significant difference in implantation rate and offspring delivery rate when using triple anesthetics compared to isoflurane.

Fetal birth rate is significantly reduced without postoperative warming

Production of pups following genetically modified mice embryo transfer.

On hot plate (40℃) after surgery	Genetic background	Embryo Stage	No.of 2-cell embryos transferred	No. of pups	% of pups
−	C57BL/6	2−cell	266	104	38.8±3.0
＋	C57BL/6	2−cell	306	162	53.3±4.6*

*P<0.05, Mean±SE

Due to their small body size, mice are prone to hypothermia during general anesthesia, requiring warming during and after surgery.

Ⅲ 型

Heater mat, Natsume Seisakusho

Body temperature change after awakening

Using 7-week-old ICR females, changes in body temperature were measured after returning to the cage after awakening in isoflurane and M/M/B anesthesia groups. The isoflurane anesthesia group was induced with 3% isoflurane, maintained at 2% isoflurane for 20 minutes, returned to the cage after recovery of the righting reflex, and rectal temperature was measured every 10 minutes. In the M/M/B anesthesia group, M/M/B was administered subcutaneously to it, Aantisedan was administered 20 minutes after the loss of righting reflex, and rectal temperature was measured every 10 minutes after recovery of the righting reflex (Fig. 4).As a result, the body temperature of the isoflurane anesthesia group decreased by about 0.8°C in about 20 minutes after waking up, and returned to the original body temperature in about 40 minutes. In addition, the body temperature of the M/M/B anesthesia group decreased by about 4.3°C 120 minutes after awakening, and returned to the original body temperature in about 300 minutes (Fig. 5). It was shown that isoflurane caused less hypothermia after awakening.

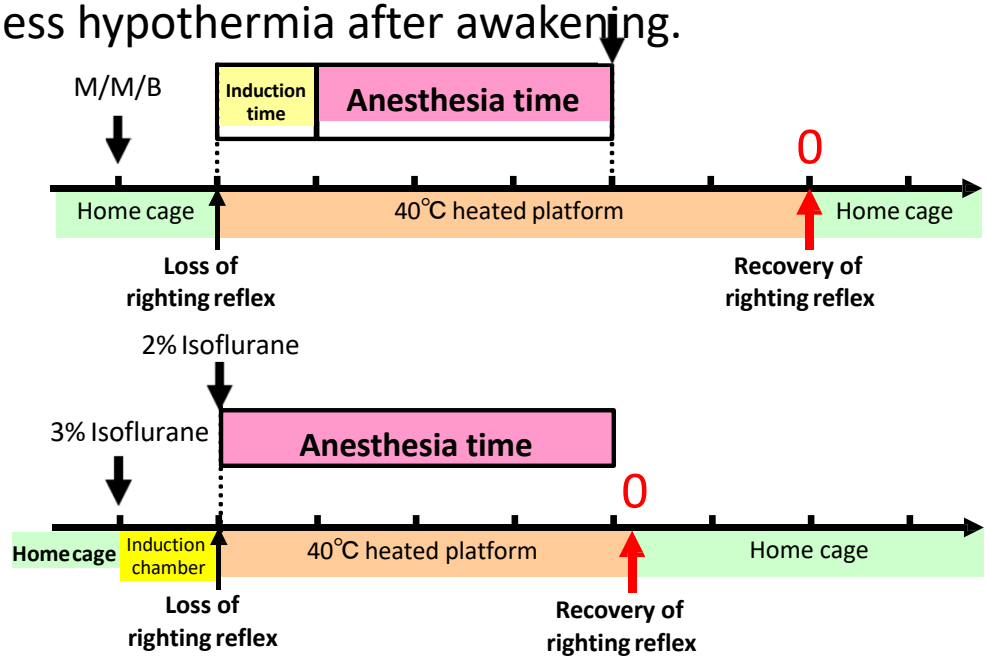


Fig. 4. Time course of anesthesia

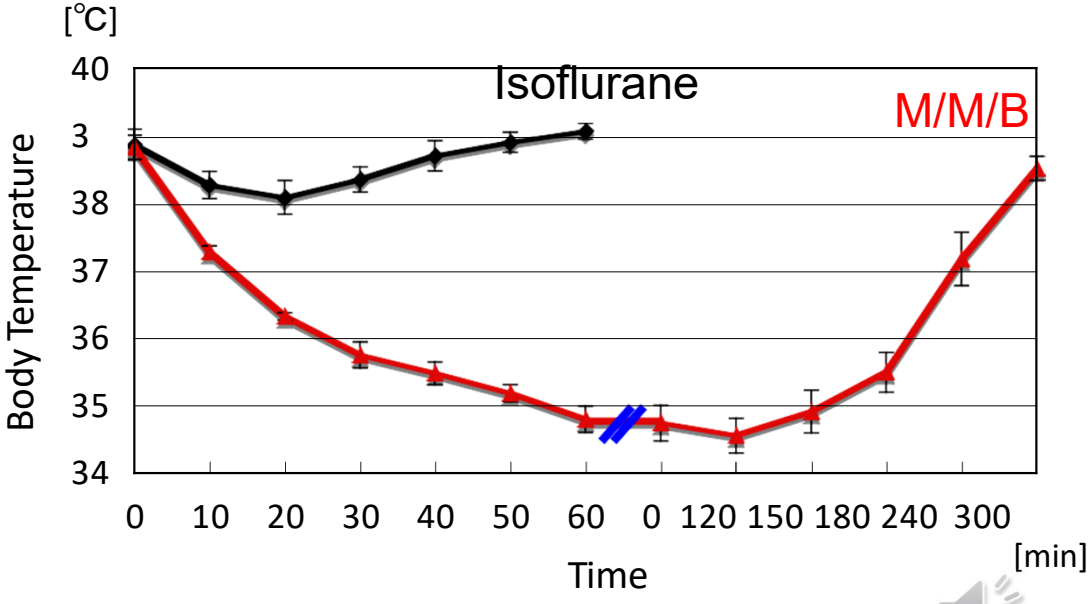


Fig 5. Body temperature after awakening

Characteristics of α2 adrenergic receptor agonists (medetomidine, xylazine, etc.)

[pros]

1,620 : 1 (medetomidine) 160 : 1 (xylazine)

α2/α1 selectivity

・Very strong sedative.

・It exhibits not only sedative effects but also analgesic and muscle relaxant effects.

・It works additively and synergistically with other sedatives, narcotics, and anesthetics, thus reducing the dose of each other.

・Respiratory depression is generally mild.

・Antagonist (Atipamezole) can be rapidly discontinued.

[cons]

・Since α2-adrenergic receptors are distributed throughout the body, they exhibit various effects other than sedative effects.

・Transient elevation of blood pressure due to peripheral vasoconstriction.

・Strong bradycardia (baroreflex-parasympathetic stimulation in response to increased blood pressure).

・Body temperature tends to drop (especially in cats).

・Increased blood sugar level (suppression of insulin secretion from pancreatic ß cells)

・Diuretic

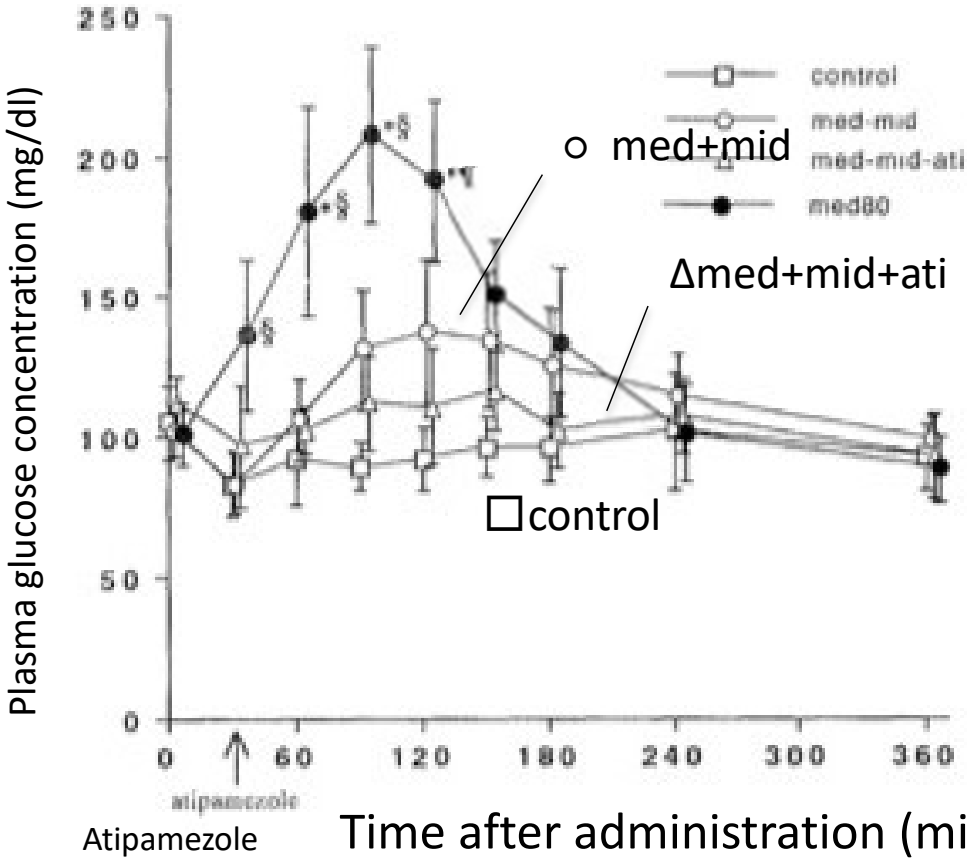
Increasing the amount of medetomidine also enhances side effects

Supervised by Nobuo Sasaki, Veterinary Clinical Anesthesiology, Gakusosha, partially modified

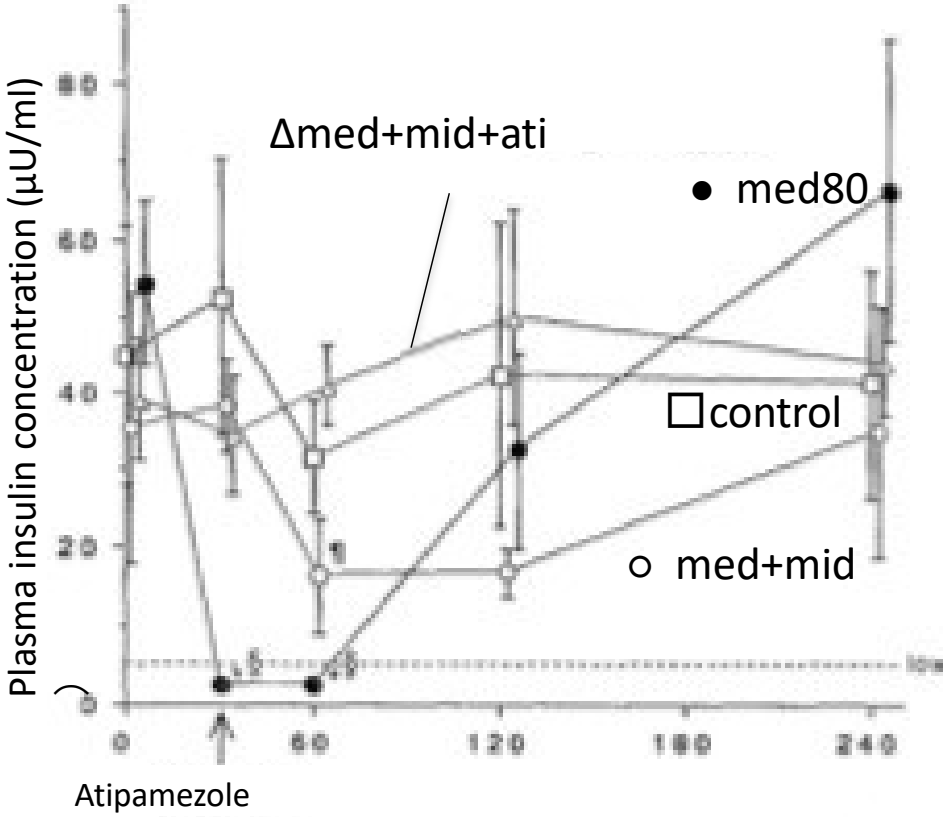
Effect of medetomidine to increase blood glucose level

Medetomidine in Laboratory Pigs: Effect of Midazolam on Blood Glucose and Blood Insulin Concentrations.

Blood glucose level after anesthesia administration



Insulin concentration after anesthesia administration



● med80; Medetomidine 80ug/Kg BW

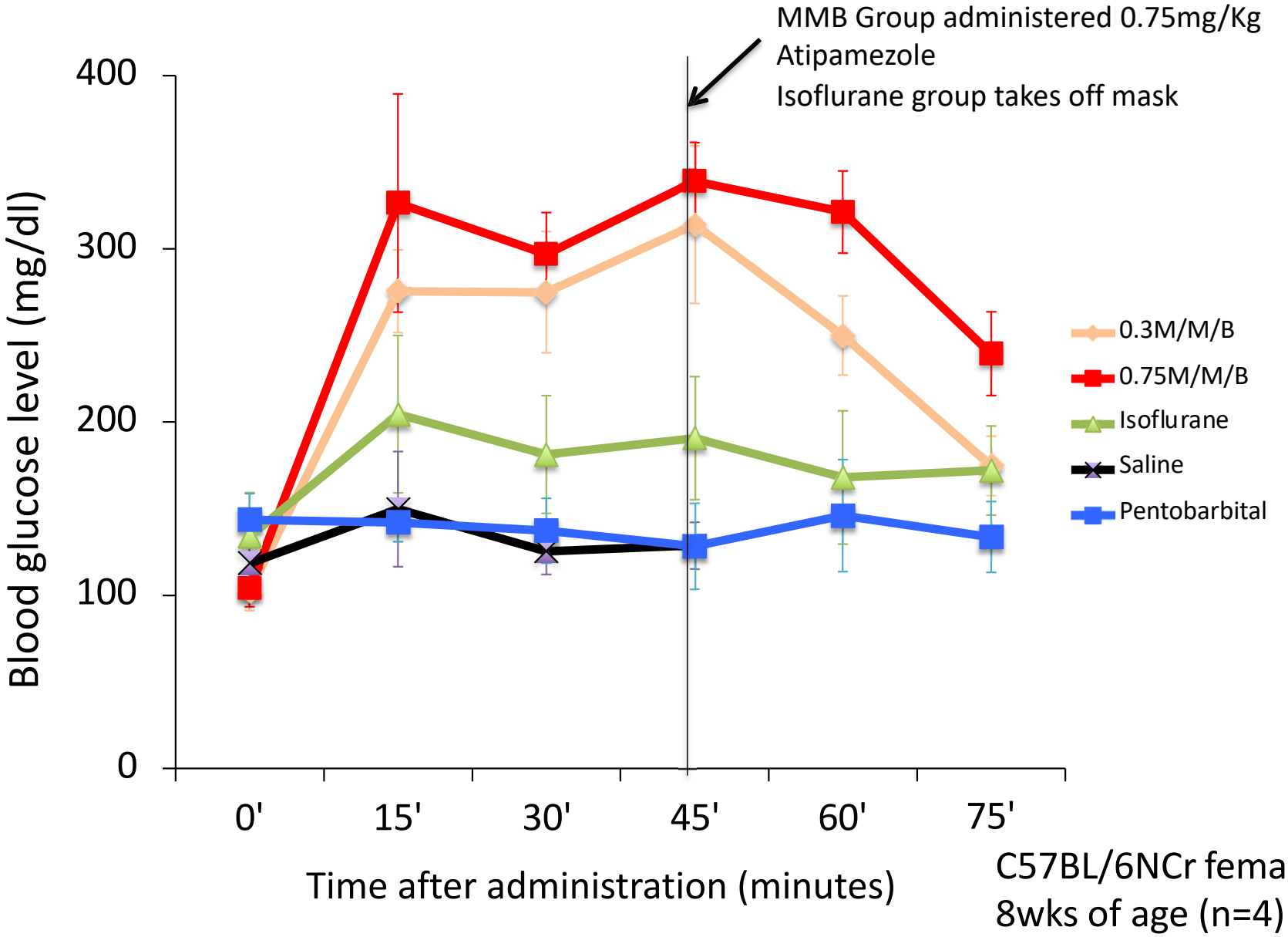
○ med+mid; Medetomidine 40ug/Kg + Midazolam 0.2mg/kg BW

Δmed+mid+ati; Medetomidine 40ug/Kg + Midazolam 0.2mg/kg + Atipamezole 160ug/Kg

☐control; saline administration

Nishimura, R et al, *JVMS*, 56, 559-561 (1994)

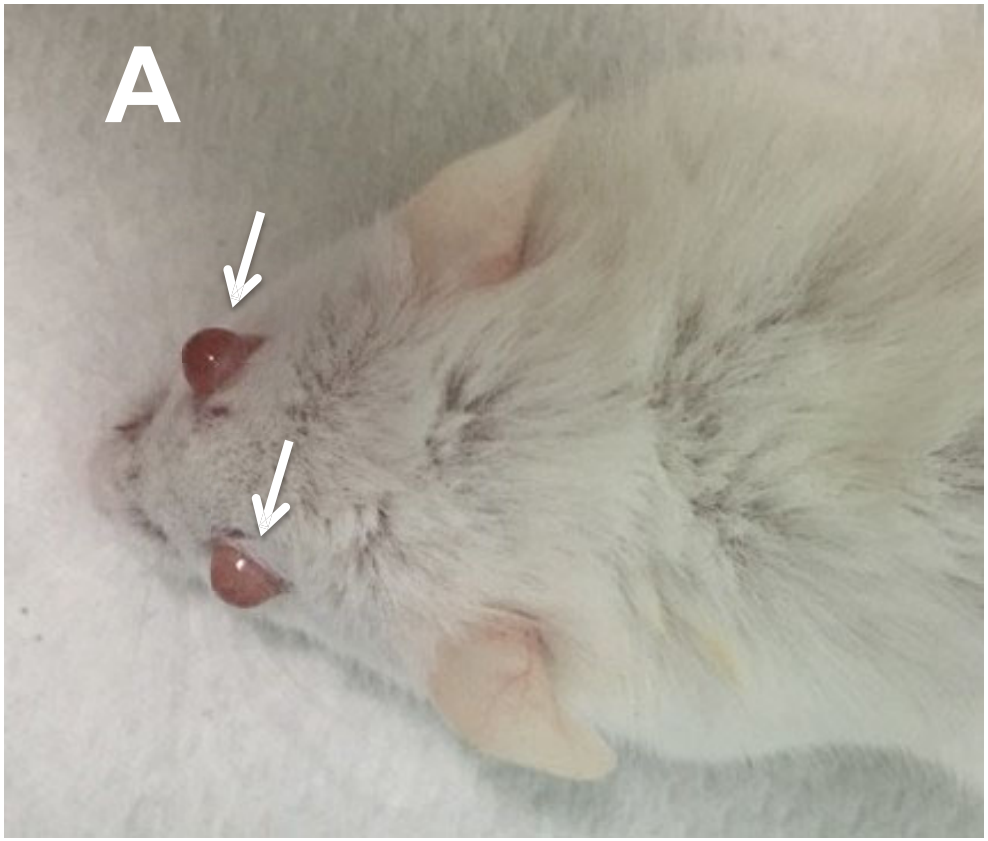
Effect of each anesthetic on blood glucose level

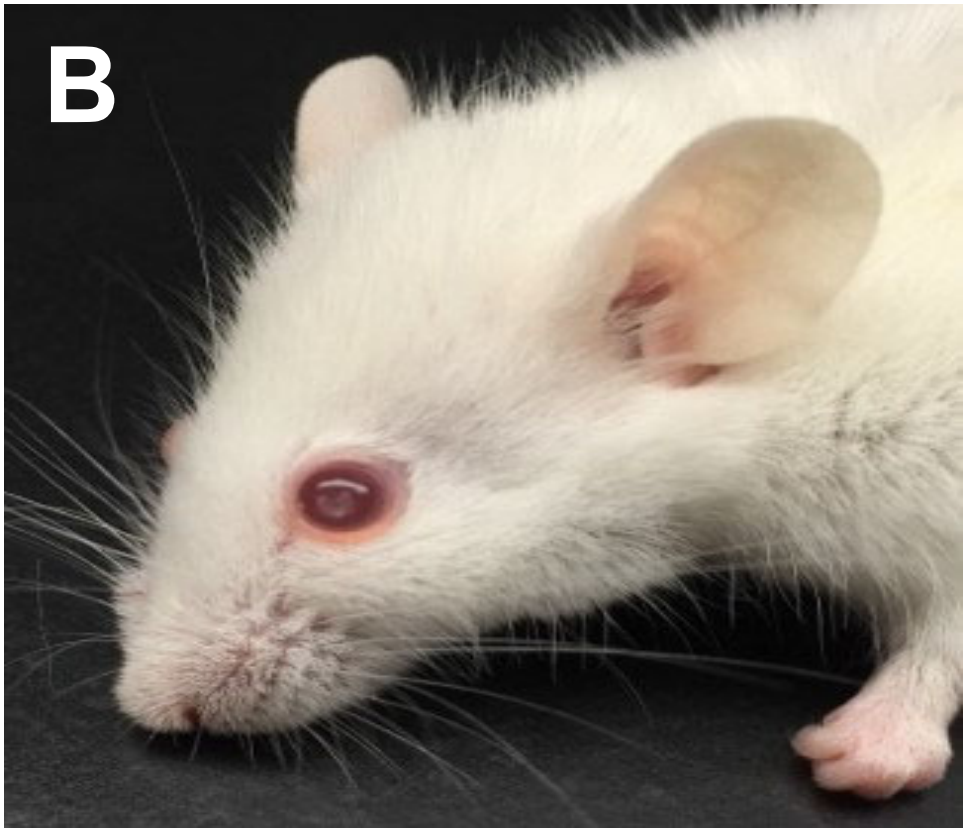


C57BL/6NCr female,
8wks of age (n=4)

In the MMB group, the blood glucose level significantly increased after administration of anesthesia

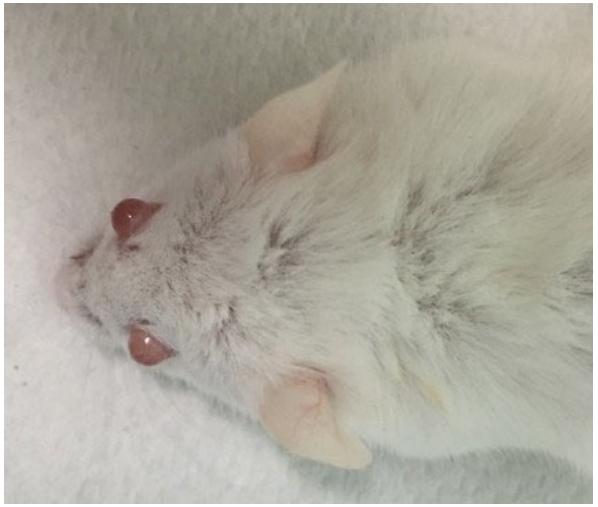
Effects of triple anesthetic (0.75M/M/B) on eyes



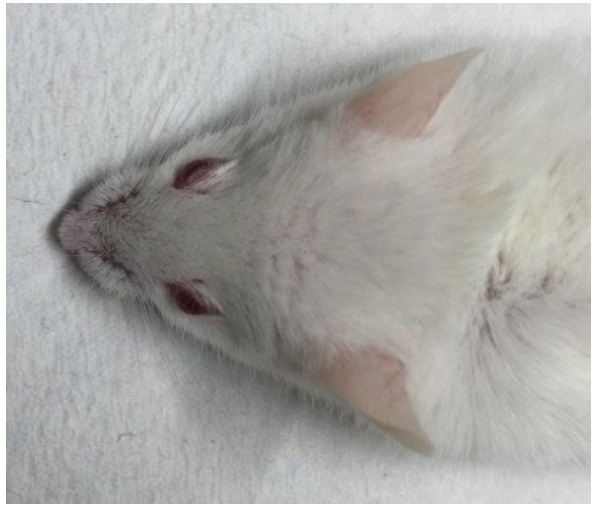


(A) Protruding eyes were observed in all cases (5 minutes after administration, arrow).

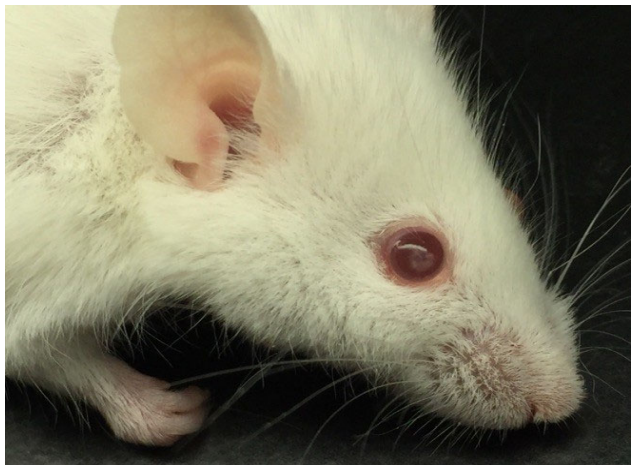
(B) Acute reversible cataract seen after triple anesthetic administration. Both proptosis and cataract recovered in all cases after administration of the antagonist.

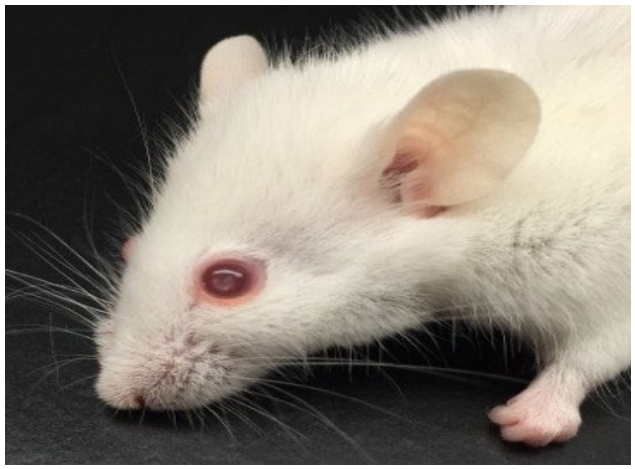


A. Protuberance associated with the administration of triple anesthesia(5 min. after administration of mixed anesthesia)

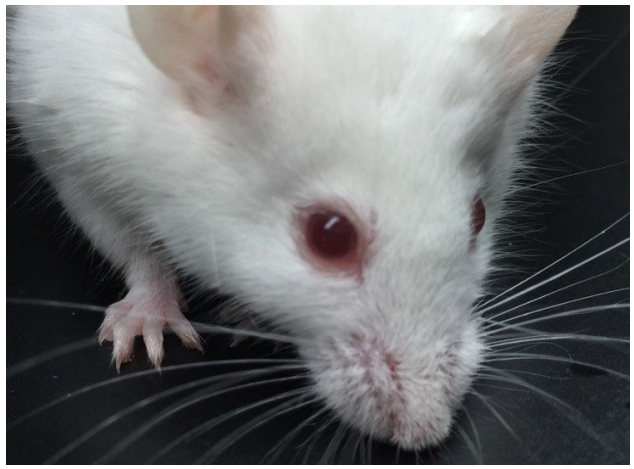


B. Eyeball retraction due to administration of α2 adrenergic receptor antagonist (5 min. after administration of atipamezole sulfate)





C. Reversible lens opacification associated with the administration of three kinds of mixed anesthetics (50 min. after administration of mixed anesthesia)



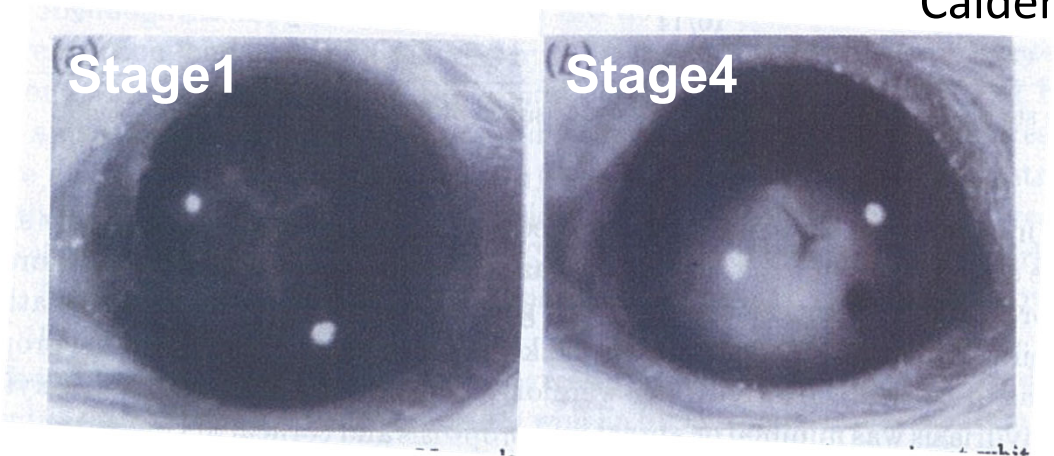
D. Improvement of lens opacity by administration of α2 adrenergic receptor antagonist (50 min. after administration of atipamezole sulfate)

LABIO 21, No.66 Oct, 2016

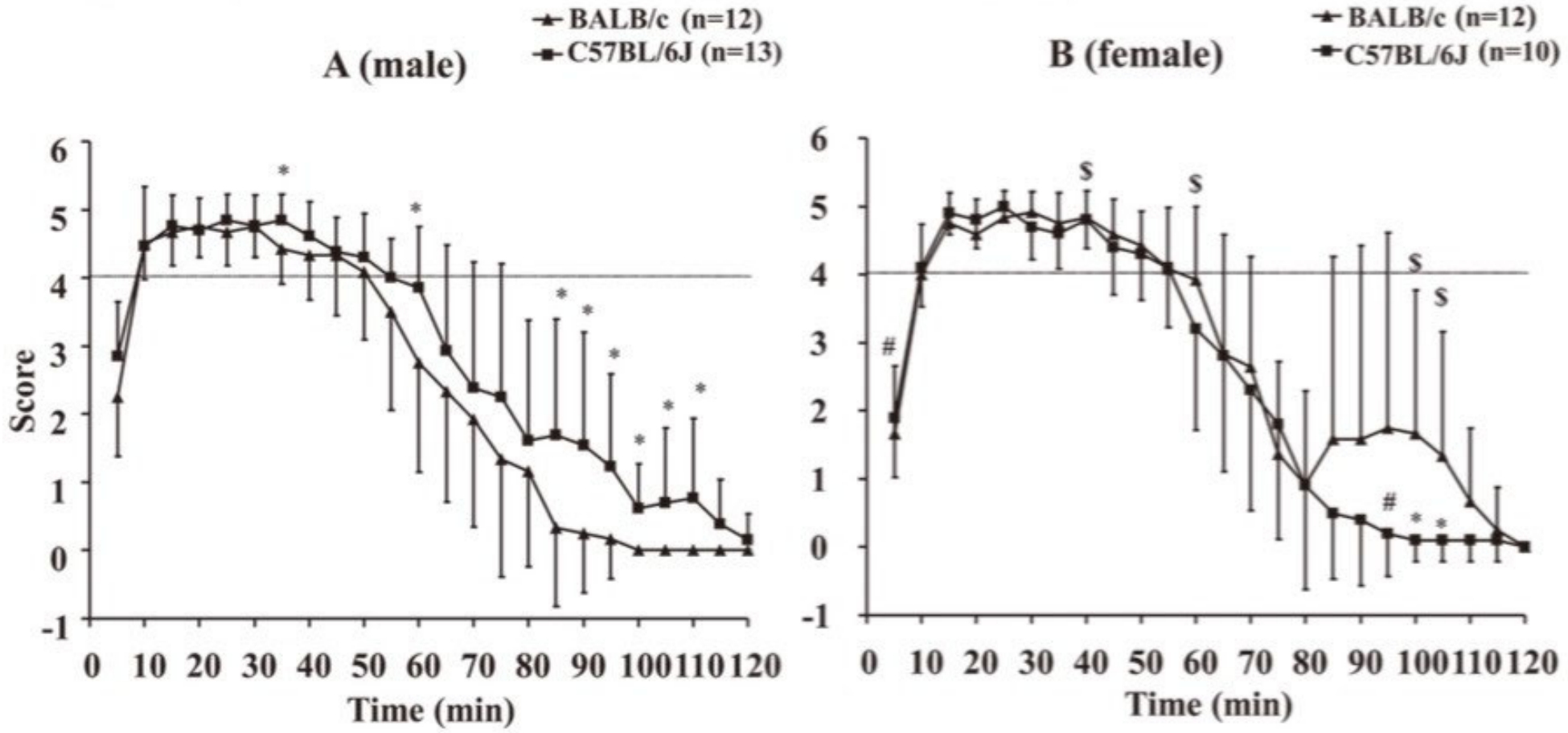
Incidence of cataract in rats and mice treated systemically with ketamine (87mg/kg) and/or xylazine (13mg/kg)

	Incidence of affected animals	eyes	Incidence of different stages of cataract 0	1	2	3	4
Rats							
Ketamine-xylazine	10/10	19/20	1	5	7	6	1
Xylazine	4/5	8/10	2	0	8	0	0
Ketamine	0/5	0/10	10	0	0	0	0
Mice							
Ketamine-xylazine	8/8	13/16	3	3	6	4	0
Xylazine	4/5	5/10	5	0	4	1	0
Ketamine	0/5	0/10	10	0	0	0	0

Calderone, L. et.al, 42, 331-337, *Exp.Eye.Res* (1986)



What is the optimal dosage for mixed anesthetics?



0.3M/M/B takes 10-15 minutes to reach surgical anesthesia time. About 15 min. after the injection, enough time for anesthesia induction is taken.

Kirihara, Y. et al, 62,173-180, *Exp.Anim*. (2013)

What is the optimal dosage for mixed anesthetics?

Blood biochemical changes in mice after administration of a mixture of three anesthetic agents

Ochiai, Y. et al, 78, 951-956 *JVMS* (2016)

Table 1. List of drugs used in this study

Group name	Drug	Product concentration	Agent dose	Administration route
Control	Saline	–	–	subcutaneous
Iso sa (+)	Saline	–	–	subcutaneou
	Isoflurane	2%	4 l/min	s inhalation
	Atipamezole*	5.0 mg/ml	1.8 mg/kg	subcutaneou
Iso sa (−)	Isoflurane	2%	4 l/min	inhalation
MMB Low	Medetomidine	1.0 mg/ml	0.45 mg/kg	subcutaneous
	Midazolam	5.0 mg/ml	6.0 mg/kg	
	Butorphanol	5.0 mg/ml	7.5 mg/kg	
	Atipamezole*	5.0 mg/ml	0.9 mg/kg	
MMB High	Medetomidine	1.0 mg/ml	0.9 mg/kg	subcutaneous
	Midazolam	5.0 mg/ml	12.0 mg/kg	
	Butorphanol	5.0 mg/ml	15.0 mg/kg	
	Atipamezole*	5.0 mg/ml	1.8 mg/kg	
Pent	Pentobarbital sodium	64.8 mg/ml	48.6 mg/kg	intraperitoneal
K / X	Ketamine	50 mg/ml	75 mg/kg	intraperitoneal
	Xylazine	20 mg/ml	10 mg/kg	
Med	Medetomidine	1.0 mg/ml	0.9 mg/kg	subcutaneous
Mid	Midazolam	5.0 mg/ml	12.0 mg/kg	
But	Butorphanol	5.0 mg/ml	15.0 mg/kg	

Atipamezole* is only administrated in Experiment 3.

1.5 times the amount

Minimum concentration for surgical operation

3 times the amount

Maximum non-lethal dose

Injectable Anesthetic - Alfaxalone

Alphaxalone, a neuroactive steroid molecule, exerts an anesthetic effect by acting on GABAA receptors like propofol. In 1971, it was formulated as an alphaxalone/alphadolone combination drug, but the solvent (castor oil derivative) used caused severe allergic reactions and was discontinued. However, in the latter half of the 1990s, a highly safe formulation using cyclodextrin as a solvent was developed and approved in Japan as an anesthetic inducer for dogs and cats.

【Advantage】

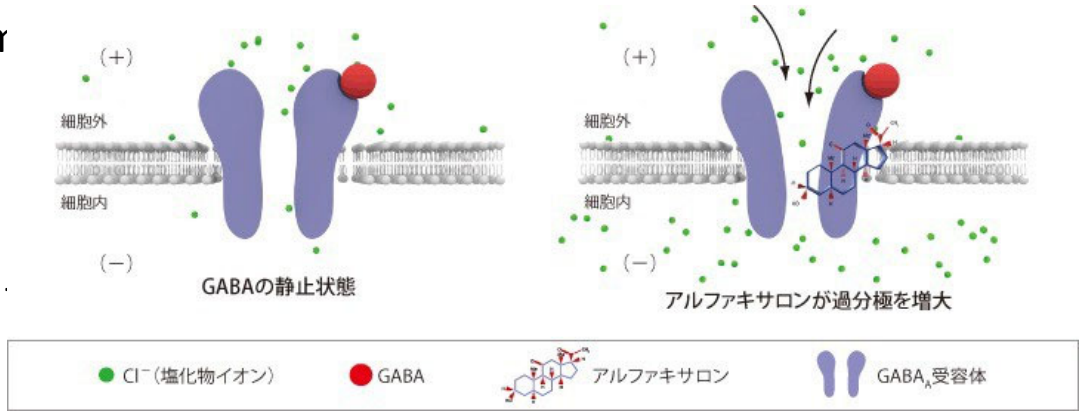
Rapid onset and awakening No irritation

Wide safety margin, little respiratory depression, m relaxation

Veterinary drug

[Use and usage]

Long-term maintenance anesthesia is possible due metabolism



Immobilization by intramuscular administration

Intravenous propo
Easier to use than foam

https://www.meiji-seika-pharma.co.jp/animalhealth/ca/doctor/alfaxan/

Anesthetic effect of a mixture of alfaxalone, medetomidine, and butorphanol for inducing surgical anesthesia in ICR, BALB/c, and C57BL/6 mouse strains

Tsukamoto Y et al, *J. Vet. Med. Sci.* 81(6): 937–945, 2019 doi: 10.1292/jvms.18-0712

Medetomidine, alfaxalone, and butorphanol are useful for anesthesia in ICR, BALB/c, and C57BL/6.

MMB: Medetomidine 0.3mg/kg, Midazolam 4mg/kg, Butorphanol 5mg/kg

MBA30: Medetomidine 0.3mg/kg, Alfaxalone 30mg/kg Butorphanol 5mg/kg,