Establishment of safe anesthesia method for preventing hypothermia and hyperglycemia induced by medetomidine-midazolam-butorphanol in mice

Abstract of Doctoral Thesis

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Injectable anesthesia is easy to administer and eliminates the need to deal with an anesthetic machine required for inhalation anesthesia. The anesthetic combination (MMB) of three injectable anesthetics, medetomidine (Me), midazolam (Mi), and butorphanol (Bu), has been commonly used in mice. A dose of MMB (Me/Mi/Bu = 0.3/4.0/5.0 mg/kg) provides sufficient anesthesia for 40–50 min in mice. In addition, atipamezole (Ati) is available to reverse MMB anesthesia. However, hypothermia, a common adverse effect of anesthesia in laboratory animals, has also been associated with MMB anesthesia in mice. Small rodents, such as mice, are particularly susceptible to hypothermia during anesthetic events. Therefore, the animals require additional thermal support from external heating devices during and after anesthesia. In general, the recovery time from anesthesia is typically longer with injectable anesthetics than with inhalants; however, the duration of thermal support has been typically limited to about 1 h from the administration of anesthesia. In addition to hypothermia, hyperglycemia has been observed in mice under MMB anesthesia. As anesthesia is often used in the study of glucose metabolism, MMB anesthesia may be contraindicated as it can influence the blood glucose level (BGL) of mice. Pentobarbital sodium (Pent), a short-acting barbiturate widely used as an anesthetic in rodents, shows few effects on glucose metabolism but has a poor analgesic effect. Although Pent can be commercially obtained, it is classified as a non-pharmaceutical-grade compound and, as such, is unsuitable as an anesthetic agent. Secobarbital (Seco), which is a pharmaceutical-grade barbiturate, is a known substitute for MMB or Pent. This study (1) compared the levels of hypothermia induced by injectable MMB anesthesia with inhalant isoflurane (ISO) anesthesia and investigated the component of MMB responsible for hypothermia, (2) determined the adequate duration of thermal support in mice after anesthesia administration and the doses of Ati and MMB necessary to prevent hypothermia, and (3) evaluated the effects of Seco on BGLs and body temperature in mice.

All procedures in this study were approved by the provisions of Nippon Veterinary and Life Science University (Approval Nos. 28S-62, 29K-25, 30K-26, 2019K-14, 2020K-39, and 2021K-49).

Animals

ICR male mice aged older than 9 weeks were used in this study.

Body temperature-measuring device

Mice were implanted with a small device in advance that allowed for the continuous noninvasive measurement of core body temperature.

Thermal support

External thermal support was provided by warming cages with a hot plate for 10 min during the induction period of anesthesia. The hot plate was set at 46°C on the surface of the warming cage to maintain approximately 37°C. Normothermia was maintained for 30 min during anesthesia using a

heating pad. The heating pad was controlled at 37°C, assuming a surgical operative period of 30 min. The warming cage was used for additional thermal support for 1, 2, 3, and 5 h. At the end of the thermal support, mice were returned to their home cages.

Anesthetic score

We evaluated anesthetic depth by scoring based on presence = 1 or absence = 0 of reflexes in the mice measured every 5 min for 40 min. The 5-point reflexes evaluated were loss of (1) the righting reflex, (2) the pedal withdrawal reflex in each forelimb and (3) hindlimb, (4) the tail pinch reflex, and (5) the corneal reflex. The scores obtained from the 5-point reflex were summed, and a score of 4–5 was accepted as a surgical anesthetic plane.

(1) Hypothermia levels after anesthesia and the MMB anesthetic component causing hypothermia (without thermal support) (Chapter 3)

1-1) Levels of hypothermia induced by MMB and ISO anesthesia

We compared the decrease in body temperature associated with MMB and ISO anesthesia. Mice implanted with a temperature-monitoring device were anesthetized with MMB (Me/Mi/Bu = 0.3/4.0/5.0 mg/kg) or ISO (induction: 5% and maintenance: 2%, with an O_2 flow rate of 2 L/min) for 40 min. At 40 min, MMB anesthesia was reversed by administrating Ati (0.3 mg/kg), and ISO inhalation was discontinued. The mice were placed back into their home cages. Both anesthetic groups experienced significant hypothermia ($p < 0.01$). In addition, recovery to normothermia in the MMB group was significantly delayed ($p < 0.01$) compared with the ISO group.

1-2) Body temperature after administration of Me, Mi, Bu, and Mi/Bu

We investigated the key component in MMB responsible for hypothermia in mice and compared the change in body temperature after the administration of Me (0.3 mg/kg), Mi (4.0 mg/kg), Bu (5.0 mg/kg), and Mi/Bu (4.0/5.0 mg/kg) without thermal support. The body temperature of the Me group was the lowest significantly compared to the other groups ($p < 0.01$).

2) Methods of preventing hypothermia induced by MMB (with thermal support) (Chapter 4)

2-1) Investigation of duration of thermal support required to prevent hypothermia induced by anesthesia

We compared the change in body temperature of mice after the end of thermal support for 1 (both groups), 2, 3, and 5 (MMB group only) h from the administration of anesthesia. The duration of thermal support required to prevent hypothermia induced by MMB and ISO anesthesia was assessed. Mice in the ISO group maintained normothermia with thermal support for 1 h. In the MMB group, the body temperature of mice decreased, and hypothermia occurred within 60 min after the end of thermal support for 1 and 2 h. However, the MMB mice maintained their body temperature within

the normothermic range with thermal supports for 3 and 5 h. Several hypothermic mice were observed at a specific frequency with thermal support for 3 h but not at all for 5 h.

2-2) Effects of Ati on hypothermia induced by MMB and blood biochemical parameters

We investigated the antagonism of Ati for hypothermia induced by MMB. The mice implanted with a body temperature-measuring device were anesthetized with MMB. After 40 min, the mice were injected with Ati 0.3, 0.6, 1.2, and 2.4 mg/kg. In addition, the mice were treated with thermal support for 2 h from the administration of MMB. Plasma levels of creatinine phosphokinase (CPK), aspartate transaminase (AST), and alanine transaminase (ALT) were assessed in mice administered saline or high doses of Ati (1.2 and 2.4 mg/kg). Blood was collected by decapitation at 1 and 3 h after the injection. The activities of CPK, AST, and ALT in the plasma samples obtained were measured by using a chemical analyzer. The administration of Ati to mice anesthetized with MMB prevented hypothermia in a dose-dependent manner, and recovery time to normothermia was shortened in a dose-dependent manner. Plasma concentrations of CPK and AST were moderately changed.

2-3) Dose determination for MMB anesthesia for preventing hypothermia

The mice implanted with a body temperature–measuring device were administered Me (0.1, 0.2, and 0.3 mg/kg) and treated with thermal support for 2 h. Changes in body temperature after thermal support were compared in each group. The body temperature in the Me 0.3 group significantly decreased between 20 and 180 min from the end of 2-h thermal support compared with the Me 0.2 and 0.1 groups ($p < 0.01$). In addition, anesthetic scores were compared for 40 min in different MMB dose groups (Me/Mi/Bu = 0.3/4.0/5.0 (original), 0.3/6.0/7.5 (higher than original), 0.15/6.0/7.5, 0.15/6.0/10, 0.2/6.0/7.5, and 0.2/6.0/10 (four modified doses) mg/kg). In the four modified doses of MMB, the administration of 0.2/6.0/10 mg/kg produced a surgical anesthetic depth (total scores of 4 and 5) between 10 and 40 min. The dose of 0.2/6.0/10 mg/kg induced a surgical anesthetic depth similar to the doses of 0.3/4.0/5.0 and 0.3/6.0/7.5 mg/kg. The body temperature after the end of thermal support for 2 h was significantly decreased compared to normothermia with MMB 0.3/4.0/5.0 mg/kg, but the significant decrease in body temperature was not observed with MMB 0.2/6.0/10 mg/kg. In addition, the recovery time to return to normothermia was dramatically improved with MMB 0.2/6.0/10 mg/kg compared with 0.3/4.0/5.0 mg/kg ($p < 0.01$).

3) Evaluation of Seco as an alternative injectable anesthetic to MMB (Chapter 5)

3-1) BGL evaluation following anesthesia

We evaluated the BGL after the administration of each anesthetic. The mice were administered saline, MMB 0.3/4.0/5.0 (original) and 0.2/6.0/10 (improved) mg/kg, Pent 75 mg/kg, and Seco 75 mg/kg. Blood was collected by decapitation 15 min after the administration of each drug, and the BGL was measured by using a glucometer. The administration of MMB significantly increased the BGL

compared with saline, Pent, and Seco. There were no significant differences in the BGL between saline and the barbiturates (Pent and Seco).

3-2) Dose determination for Seco anesthesia

We evaluated the anesthetic score with Seco for 40 min. Mice were provided with thermal support, as described previously. The mice were treated with Seco (50, 75, and 100 mg/kg), Pent (50, 75, and 100 mg/kg), and Seco and Bu (SB) (Seco 75 mg/kg injected 10 min after the injection of Bu 5.0 mg/kg). The administration of SB produced a surgical anesthetic depth, whereas the other anesthetic groups did not achieve a surgical anesthetic plane.

3-3) Evaluation of BGL and body temperature after administration of SB (compared with MMB)

We compared changes in the BGL after the administration of MMB 0.3/4.0/5.0 and SB (Seco 75 mg/kg injected 10 min after the injection of Bu 5.0 mg/kg). Blood was collected by decapitation 15 min after the injection, and the BGL was measured by using a glucometer. We also compared the change in body temperature of mice with thermal support for 2 h after the administration of MMB 0.3/4.0/5.0 mg/kg and SB. The administration of MMB significantly increased the BGL compared with SB ($p < 0.01$). In addition, the body temperature after the end of thermal support was significantly decreased in the MMB group compared with the SB group ($p < 0.01$).

4) Conclusions (Chapter 6)

In conclusion, (1) the α_2-agonist Me, a component in MMB, is most likely responsible for inducing hypothermia in mice. (2) The 5-h duration of thermal support in the MMB group and 1-h duration in the ISO group completely prevented hypothermia. The antagonism of Ati within the proper dose range effectively promoted recovery from MMB-induced hypothermia. MMB at our recommended dose of 0.2/6.0/10 mg/kg provides anesthetic effects for 40 min and normothermia after 2 h of thermal support. (3) The administration of Seco alone did not induce a surgical anesthetic depth in mice, but the combination of SB maintained a surgical anesthetic depth for 40 min. In MMB groups, the BGL significantly increased compared with Pent, Seco, and SB groups. In addition to the mild effects of SB on BGL, hypothermia was blocked by thermal support for 2 h in the SB group. This study provides suitable anesthesia methods for preventing hypothermia and hyperglycemia in mice.