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

Summary of Doctoral Thesis

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A combination of three anesthetics (MMB), namely medetomidine (Me), midazolam (Mi), and butorphanol (Bu), is commonly used as injectable anesthesia in mice. An original dose of MMB (Me/Mi/Bu = 0.3/4.0/5.0 mg/kg) provides a sufficient anesthetic duration of 40–50 min in mice. In addition, atipamezole (Ati) is available for the reversal of MMB anesthesia. However, severe hypothermia has been observed in mice as an adverse effect of MMB anesthesia. Hypothermia during anesthetic events is a common adverse effect of anesthesia in laboratory animals. In particular, small rodents, such as mice, are susceptible to hypothermia during anesthetic events. Therefore, these animals need an additional thermal support from external heating devices during and after anesthesia. In general, the time of recovery from anesthesia is typically longer in the case of injectable anesthesia than that in the case of inhalant anesthesia. However, the duration of thermal support has been almost limited to about 1 h from the time of administration of anesthesia. In addition to hypothermia, hyperglycemia has been observed in mice under MMB anesthesia. Anesthesia is often used for studying glucose metabolism; therefore, the use of MMB anesthesia may influence the blood glucose level (BGL) of mice. Pentobarbital sodium (Pent), which is a short-acting barbiturate widely used as anesthesia in rodents, has a poor analgesic effect and shows few effects on glucose metabolism. Although Pent can be commercially obtained, it is classified as a non-pharmaceutical-grade compound and is not suitable as an anesthetic agent. Secobarbital (Seco), which is a pharmaceutical-grade barbiturate, is a known substitute for MMB or Pent. In the present study, we aimed 1) to compare the levels of hypothermia induced by injectable anesthesia with MMB and inhalant anesthesia with isoflurane (ISO) and investigate the anesthetic component of MMB responsible for causing hypothermia, 2) to find the adequate duration of thermal support in mice after administration of anesthesia and provide the doses of Ati and MMB mixture for preventing hypothermia, and 3) to evaluate the effects of Seco on BGLs and body temperature in mice. All procedures in this study have been 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). The results of this study revealed that 1) α_2-agonist Me, a component of MMB, is most likely to induce hypothermia. 2) A 5-

h thermal support completely prevented hypothermia in the MMB group and a 1-h-support prevented hypothermia in the ISO group. The antagonism of Ati within the proper dose range is effective in promoting recovery from MMB-induced hypothermia, and MMB at our recommended dose of 0.2/6.0/10 mg/kg provides anesthetic effects for 40 min and keeps normothermia after thermal support for 2 h. 3) The administration of Seco alone did not induce surgical anesthetic depth in mice, but the anesthetic combination of Seco and Bu (SB) maintained the surgical anesthetic depth for 40 min. In the MMB group, the blood glucose levels significantly increased compared with the Pent, Seco, and SB groups. In addition to the mild effects of SB on BGL, hypothermia was blocked by a thermal support for 2 h in the SB anesthesia group. The present study provides suitable anesthesia methods for preventing hypothermia and hyperglycemia in mice.