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Neuroprotection by Anesthetics in Brain Injury Models

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Summary

The aim of the study was to compare the effect of sevoflurane and chloral hydrate on the neurological status and volume of brain damage after trauma and ischemia in experimental models of traumatic brain injury (TBI) and focal ischemic stroke (IS) induced by photothrombosis (PT).

Materials and methods. The experiments were performed on mongrel Wistar rats weighing 250–300 g (*N*=43). There were 4 groups: the Ischemia + Sevoflurane group (IS_S) (*N*=10), the Ischemia + Chloral hydrate group (IS_{CH}) (*N*=10), TBI + Sevoflurane group (TBI_S) (*N*=13), and TBI+Chloral hydrate group (TBI_{CH}) (*N*=10). Ischemic brain damage was modelled using Rose Bengal (RB) dye-induced PT, and TBI was modelled using mechanical force-induced concussion.

Results. MRI findings indicate lower volumes of brain damage (mm^3) in rats from TBI_S group compared with the TBI_{CH} group (19±5 vs. 60±5, *P*<0.0001), and in the IS_S group compared with the IS_{CH} group (9.8±1.5 vs. 21.5±2, *P*=0.0016). Moreover, there was a significant difference between IS_S and IS_{CH} groups based on the protocol assessment of neurological status on day 14 with higher scores in IS_S (11.4±1.8 vs. 4.9±2.6, *P*<0.0001).

Conclusion. Taking into account the data obtained, we recommend a careful choice of anesthesia when modeling ischemic stroke and traumatic brain injury in animals. In particular, the neuroprotective effect of sevoflurane should be taken into account in the PT and TBI models.

Keywords: neuroprotection; anesthetics; photoinduced ischemic stroke; TBI model; brain injury models; sevoflurane; chloral hydrate

Conflict of interest. The authors declare no conflict of interest.

Introduction

Models of ischemic injury, such as photochemically induced thrombosis, brain injury, middle cerebral artery occlusion, and others, are most commonly used to evaluate the neuroprotective properties of drugs. The models of dosed open brain contusion injury [1] and photochemically induced thrombosis have proven to be relevant and have a number of advantages, including low invasiveness, high reproducibility, low mortality, and the ability to control the extent of brain damage [2, 3]. To evaluate the effect of different therapeutic agents in brain injury models, it is necessary to eliminate the influence of various «confounding» factors, such as the anesthetic method or animal heterogeneity.

Anesthetics used in different experimental models possess neuroprotective properties, which impede assessing the neuroprotective effects of different therapeutic agents.

Therefore, the aim of our study was to compare the effects of sevoflurane and chloral hydrate on the neurological status and extent of brain damage in photochemically induced thrombosis (PIT) and traumatic brain injury (TBI).

Materials and Methods

Animals. Experiments were performed on 43 Wistar crossbred rats, weighing 250–300 g, maintained in a vivarium with a 12/12-hour light/dark cycle at a constant temperature (22±2°C). Animals were used in experiments in accordance with the Animal Care Protocol approved by the Animal Ethics Committee of A. N. Belozersky Research Institute of Physicochemical Biology, protocol No. 2/20, February 12, 2020.

The following four groups were distinguished:

- Ischemic stroke + sevoflurane (IS_S) group (*N*=10)
- Ischemic stroke + chloral hydrate (IS_{CH}) group (*N*=10)
- TBI+Sevoflurane (TBI_S) group (*N*=13)
- TBI+chloral hydrate (TBI_{CH}) group (*N*=10).

Anesthesia. In the IS_{CH} and TBI_{CH} groups, rats were anesthetized with chloral hydrate (300 mg/kg,

intraperitoneally). In the IS_S and TBI_S groups, animals were anesthetized with sevoflurane (5% in a gas mixture with oxygen at a flow rate of 2 L/min), and anesthesia was maintained with 2–3% sevoflurane at a flow rate of 2 L/min through a mask. Rats were placed under an infrared heating lamp for 1 h before emergence from chloral hydrate anesthesia. The duration of sevoflurane inhalation was 39.4±3.4 min and 25.5±5.4 min in the IS_S and TBI_S groups, respectively. The body temperature of the rats was maintained at 37.0±0.5°C throughout the experiment. Thermometry was performed by installing a rectal body temperature sensor, and thermoregulation was maintained in automatic mode by connecting the heating module to a thermostat and setting the limits.

PIS model. A previously described photochemical thrombosis protocol [2, 3] was used. Focal ischemic stroke was modeled in the sensorimotor cortex of the rat brain (stereotaxic coordinates from bregma: 0.5 mm distal and 2.5 mm lateral). The photosensitive dye rose Bengal was injected intravenously (3%, 40 mg/kg; Sigma-Aldrich, St. Louis, Missouri, USA). The exposed cranial region was irradiated with green light at λ=550 nm for 15 minutes.

TBI modeling. TBI modeling was performed according to the open brain contusion injury method [1].

Magnetic resonance imaging (MRI). The study was performed on day 14 after PIS and TBI on a 7 T magnetic field induction tomograph with a 105 mT/m gradient system (BioSpec 70/30, Bruker, Germany). Animals were anesthetized with isoflurane (1.5–2%) and placed in a positioning apparatus with a stereotaxic and thermoregulatory system (as described previously [4]).

A standard rat brain examination protocol was used, including acquisition of T2-weighted images [4]. The extent of brain damage was assessed by graphical analysis of the MR images and calculation of the volume of the damaged brain area in mm3 using ImageJ software (National Institutes of Health image software, Bethesda, MD, USA).

Limb placement test (LPT). Neurological status was assessed 3, 6, and 14 days after PIS and trauma. We used a well-known protocol based on a study by De Ryck et al. [5] and modified by Jolkkonen et al. [6]. The following scores were calculated for each task: 2 points, normal response; 1 point, delayed and/or incomplete response; and 0 points, no response. A total score was calculated for the seven tasks.

Statistical analysis of quantitative data was performed using GraphPad Prism 6 software (GraphPad Software). Normality of data distribution was tested using the Shapiro–Wilk test. The Student's *t*-test was used to compare the two groups if the samples compared had a normal distribution of variables. Otherwise, the Mann–Whitney test was used. Two-way ANOVA was used to compare two groups of animals at three different time points. Data are presented as *mean*±*SD*.

Results

We found a significant reduction in the extent of brain damage when sevoflurane was used as an anesthetic in a model of traumatic brain injury (Fig. *a*). MRI showed that it was almost 3 times larger with chloral hydrate (60±5 mm³) than with sevoflurane (19±5 mm³) ($P<0.0001$). There were no significant differences in neurological status between the groups (Fig. *b*).

In the PIS (ischemic stroke) model, we also observed a decrease in brain lesion when sevoflurane was used (Fig. *c*). In addition, the lesion volume (mm³) was 2-fold larger with chloral hydrate than with sevoflurane (21.5±2 vs. 9.8±1.5, $P=0.0016$). When the neurological status of rats in the IS_S and IS_{CH} groups was analyzed on day 14, significant differences in scores were found (11.4±1.8 vs. 4.9±2.6, $P<0.0001$) (Fig. *d*).

Discussion

Anesthesia used for various purposes, including clinical, veterinary, and research practice, should generally meet the following criteria: reversible loss of consciousness, akinesia, amnesia, and analgesia [7]. Data regarding the analgesic properties of chloral hydrate, a drug commonly used in animals, are conflicting. In recent years, these properties have been found to be similar to those of other commonly used anesthetics such as ketamine-xylazine, pentobarbital, and urethane [8]. In a 2023 review by Ward-Flanagan and Dickson, it was noted that chloral hydrate also produces analgesia at the cellular level through the action of its metabolite 2,2,2-trichloroethanol, which inhibits pain transmission in mammalian dorsal root ganglion neurons. In animals, this drug is most commonly administered intraperitoneally, although intravenous administration is also possible [8].

Inhalational anesthetics, including isoflurane, sevoflurane, and desflurane, are also commonly used in animals [9]. These agents are vaporized in special vaporizers, added to the carrier gas, and administered to the animals through the respiratory tract, providing rapid induction of anesthesia, short duration of action, and rapid clearance from the body after termination of supply, which is a distinct advantage over intraperitoneal anesthetics.

When studying brain injury and searching for neuroprotective drugs, it is important to use the absence of intrinsic damaging or neuroprotective effects as a criterion for the choice of anesthetic. In the presence of such properties, it is difficult to detect the proper action of the drug owing to its anesthetic effect.

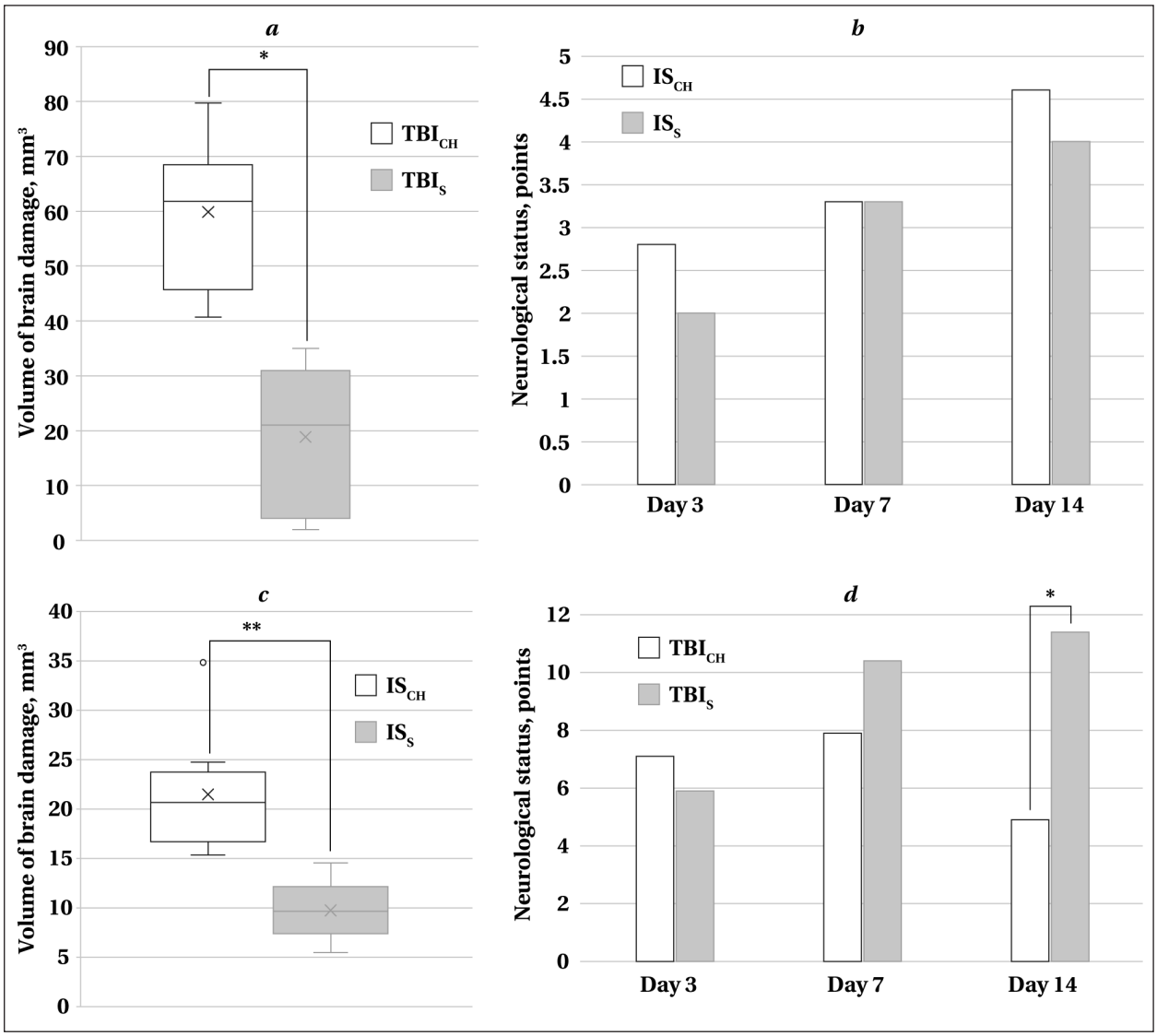

Fig. Comparison of study groups according to MRI data of the volume of brain damage *(a, c)* and scores of neurological status assessment *(b, d)*.
Note. Statistically significant differences: * — $P<0.0001$; ** — $P=0.0016$.

Literature shows that some anesthetics, such as dexmedetomidine [10] and zoletil [11], have neuroprotective properties.

In the model of PIS (ischemia) and TBI, we obtained data on the probable presence of anesthetic preconditioning properties of the anesthetic sevoflurane. Thus, sevoflurane reduced the volume of the damaged brain area in operated rats. This reduced the lesion volume observed on MRI in the group of animals operated under sevoflurane anesthesia in both models of brain injury (Fig. *a*, *c*).

In addition, the mean number of protocol scores for neurological status assessment was significantly greater in the IS_S group than in the IS_{CH} group ($P<0.0001$, Fig. *d*). Furthermore, only in the sevoflurane group were animals with maximum scores (3 rats out of 10). Although no improvement in neurological status was observed in rats treated with sevoflurane during traumatic injury in the limb placement test, the reduction in lesion size may affect the results of other neurological tests and the cellular response when studying the neuroprotective properties of drugs while using sevoflurane.

Given the data obtained, it can be concluded that it is difficult to separate the neuroprotective properties of the drugs studied from those of the anesthetic sevoflurane used in a model of TBI.

Several other studies have also described the neuroprotective effects of sevoflurane in a model of TBI [12]. TBI was induced in rats who were anesthetized with 3% sodium pentobarbital (50 mg/kg) and then received inhalation of sevoflurane for 1 h. Sevoflurane reduced brain edema, improved neurological parameters, and decreased neuronal apop-

tosis and autophagy in rats with TBI. Sevoflurane postconditioning activates fibroblast growth factor 2 (FGF2), which protects the blood-brain barrier from damage during TBI in mice [13].

There is also evidence that sevoflurane may exert neuroprotective effects in models of focal or global cerebral ischemia [14–17]. The neuroprotective effects of sevoflurane have been observed when administered by inhalation prior to ischemia modeling («preconditioning» effect) [18, 19]. For example, sevoflurane-induced preconditioning 15 min or 24 h before global cerebral ischemia reduced the extent of neuronal damage in rats [18]. In *in vitro* models, sevoflurane preconditioning of rats 15 min prior to hypoxia and reoxygenation dose-dependently increased the recovery of hippocampal neuronal function after hypoxia [19].

The effect of chloral hydrate on brain injury in rats in both models cannot be completely excluded. Studies have shown the effects of chloral hydrate preconditioning in a mouse model of ischemic stroke (middle cerebral artery occlusion) [20]. Chloral hydrate was shown to reduce the extent of damage and improve neurological status. A possible mechanism of action is an increase in the expression of annexin A1, an anti-inflammatory factor [20]. Chloral hydrate also had neuroprotective properties when used in combination with ischemic preconditioning [11, 21]. Despite these findings, sevoflurane was found to be more neuroprotective than chloral hydrate. However, the mechanism underlying this effect is not fully understood.

Conclusion

Sevoflurane is more neuroprotective than chloral hydrate in rat models of brain injury. The feasibility of using sevoflurane as an anesthetic agent when evaluating the neuroprotective potential of other therapeutic agents is questionable. Replacing sevoflurane with chloral hydrate may be a reasonable option.

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