Li et al. BMC Anesthesiology (2025) 25:109
https://doi.org/10.1186/s12871-025-02956-9

BMC Anesthesiology

RESEARCH

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Microtubule-modulating drugs alter sensitivity to isoflurane in mice

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Abstract

Background Microtubules (MTs) have been postulated as one of the molecular targets underlying loss of consciousness induced by inhalational anesthetics. Microtubule-targeting chemotherapy drugs and opioids affect MT stability and function. However, the impact of prolonged administration of these drugs on anesthetic potency and anesthesia induction and emergence times remain unelucidated.

Methods Epothilone D, paclitaxel, vinblastine or opioid morphine were administered alone for a prolonged period (> 2 weeks) to male CD1 mice and their sensitivity to incremental concentrations of isoflurane were examined using loss of righting reflex (LORR) response as a measure of sensivity. The induction and emergence time after administration and termination of fixed concentration of isoflurance (1.2%) were also assessed.

Results Compared with saline treatment, epothilone D and vinblastine induced a leftward (more sensitive) shift of LORR response curves (95% confidence intervals for EC50: epothilone D, 0.75[0.73, 0.77] vs. saline, 0.97[0.96, 0.98]; vinblastine, 0.74[0.73, 0.75] vs. saline, 0.98[0.97, 0.99]). In contrast, morphine caused a rightward (more resistant) LORR response curve (morphine, 1.16[1.15, 1.17] vs. saline, 0.97[0.96, 0.98]), while paclitaxel produced a marginal but significant rightward shift of LORR (paclitaxel, 1.05[1.03, 1.06] vs. saline, 0.98[0.97, 0.99]). At concentration of 1.2% isoflurane, morphine treatment prolonged (275 ± 50) and vinblastine treatment reduced (96.5 ± 26) the anesthetic induction latency (in second) relative to saline treatment (211 ± 39). The latency of emergence from anesthesia was shorter in morphine (58 ± 20) and vinblastine-treated (98 ± 43) mice compared to saline (176 ± 50) treatment. The induction or emergence latencies of epothilone D or paclitaxel treatment did not differ from saline treatment between groups.

Conclusions Microtubule-modulating drugs can affect not only sensitivity but also induction and emergence times to inhalational anesthetic isoflurane in mice. This study highlights a possible role of MTDs in modulating anesthetic effects in disparate directions, which has implications for anesthetic concentrations that should be used for induction, maintenance and emergence of anesthesia. These findings in rodents may have relevance to the perioperative care of cancer patients who receive MT-targeting chemotherapy drugs or even opioids for pain for prolonged periods.

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Keywords Microtubule, Isoflurane, Epothilone D, Vinblastine, Paclitaxel, Morphine

Introduction

Judicious administration of inhalational anesthetics to induce and maintain surgical anesthesia is critical for the success of surgery, minimize intraoperative complications and post-surgical recovery [1]. Certain heredity traits, acquired diseases or co-administered drugs alter the response to inhalational anesthetics [2, 3]. Although multiple molecular and cellular mechanisms for general anesthesia have been proposed [4, 5], the mechanism of action of inhaled anesthetics to induce reversible loss of consciousness during general anesthesia remains unclear [6]. Studies have shown that anesthetics bind to brain microtubules [7, 8] and affect their stability [9]. Composed of α- and β-tubulin subunits, microtubules are highly abundant in neurons in the central nervous system [10] and participate in the maintenance of neuronal structure and function [10, 11]. The levels and types of α-tubulin post-translational modifications (PTMs) are associated with the stability of the microtubules. A recent study using Xenon isotopes [8] lent support to microtubule quantum channels theory [12, 13] for anesthetic action. These findings raise the question whether drugs with microtubule-modulating properties alter the pharmacodynamics of inhaled anesthetics and the answer may have a significant impact on safety, efficacy of anesthesia, and mitigation of complications.

Microtubule-targeting drugs (MTDs) are among the most important cancer treatment tools based on their ability to inhibit signaling events important for carcinogenesis as well as to inhibit mitosis. Understanding the impact of MTDs on pharmacodynamics of inhalational anesthetics will provide more insight into the role of microtubules in general anesthesia and may help improve perioperative care for cancer patients. We will examine three important MTDs which are widely used for cancer treatment: paclitaxel, vinblastine, and epothiolone D. Taxanes (e.g. paclitaxel), a group of antineoplastic agents that stabilize microtubules, are commonly used for non-small cell lung cancer, ovarian cancer, and breast cancer treatment. A retrospective clinical study showed that female breast cancer patients who received taxane neoadjuvant therapy prior to surgery were partially resistant to general anesthetic compared with those who had not received chemotherapy prior to surgery [14]. Breast, colorectal and lung cancers are among the top ten most prevalent cancers in the US in 2019 [15], for which neoadjuvant chemotherapy (e.g., paclitaxel) is used to shrink invasive tumors to improve surgical options and survival rates [16]. Epothiolones, a class of compound derived from bacteria, are novel potent microtubule stabilizing agents which prevent cancer cell dividing by binding to microbutules. Epothilone D (generic name: utidelone) was approved in China in 2021 for the treatment of metastatic breast cancer. Vinblastine is one of the oldest chemotherapeutic agents still in use to treat a variety of cancers, including lymphoma, melanoma, breast cancer, testicular cancer, etc. Vinblastine depolymerizes microtubules preventing cancer cells from dividing. While vinblastine is a microtubule destabilizer [17], paclitaxel is a taxane, which stabilize microtubules [17]. Both paclitaxel and vinblastine have low BBB permeability [18, 19], but nevertheless, behavioral studies have documented their impact on cognitive function in mice [20, 21]. Paclitaxel, epothiolone D, and vinblstaine kill cancer cell by binding to microtubules. These drugs interact with microtubules differently at molecular level, thereby making them useful tools to assess the relevance of microtubules as a candidate target of anesthethic action.

Although microtubules are not a specific target of opioid action (analgesia), protein analyses have shown that opioids modulate brain microtubule expression [22, 23]. Substance use disorder patients or patients receiving prolonged intermittent opioids for medical reasons may require urgent or elective surgeries [24]. Many cancer patients receive opioids for prolonged periods for pain relief. Moreover, patients suffering from major burn injury usually receive repetitive large doses of opioids to alleviate pain [25]. Up to now, clinical studies have focused on postsurgical pain management for these patients [26]. Given microtubule-modulating effect of opioids, it is imperative to investigate the impact of long-term opioids on the potency of inhaled anesthetics. The insight gained will help enhance the safe administration of inhaled anesthetics during surgery in patients with pre-existing opioid use disorders or already on opioids for acute and/or chronic pain.

In this study, we examined the effects of four MTDs on isoflurane anesthesia in mice. Morphine was used as a prototypic opioid. Three chemotherapeutic drugs, which are of clincal importance, including epothilone D, paclitaxel, and vinblastine, were tested. Sensitivity to isoflurane was assessed in mice using loss of righting reflex (LORR) as a reflector of loss of consciousness together with time for induction and emergence from anesthesia.

Materials and methods

Animals

Wildtype CD1 mice (male, 10–12 week-old) were purchased from Charles River Laboratory (Wilmington, MA) and housed at Massachusetts General Hospital (MGH) rodent housing facility. Three or four mice were housed in one cage. Mice received food and water *ad* *libum*. The temperature of the room was maintained at 20–24 °C with 40–60% humidity and a 12-hour light/darkness cycle. The experimental protocol was approved by MGH Institutional Animal Care and Use Committee. The study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

Experimental design

Sample size for behavioral study was based on the study by Miller et al. [27] Mice received opioids or microtubule-targeting agents (MTDs) for 2–5 weeks [20, 21, 28, 29] before testing for isoflurane sensitivity. Two cohorts of mice were used for the study. The first cohort mice were randomly divided into saline, morphine and epothilone D treatment groups as both drugs permeate blood brain barrier. The second cohort mice were randomly divided into saline, paclitaxel, and vinblastine treatment groups. Paclitaxel and vinblastine are less permeable to the brain.

Drug treatment

Mice were treated with morphine sulfate (McKesson, OH), epothilone D (Cayman Chemical. MI, US), paclitaxel (Sigma. MO, US) or vinblastine (Sigma. MO, US) by intraperitoneal injection (i.p.). Epothilone D (2 mg/kg) or morphine (10 mg/kg) was administered daily for two weeks. Epothilone D was dissolved in ethanol (10 mg/mL) and diluated to 0.2 mg/mL in saline for injection. Paclitaxel (20 mg/kg) was administrated three-doses (every other day) per week for four weeks. Paclitaxel was dissolved in Cremophor EL: ethanol (1:1) (6 mg/mL) and diluted in saline to 2 mg/ml for injection. Vinblastine (0.2 mg/kg, i.p.) was administered daily for five weeks. Vinblastine was dissolved in PBS, pH 7.2 at 1 mg/mL and diluted in saline to 0.02 mg/mL for injection.

Behavioral testing

Behavioral testing was performed ~ 24 h after last injection of these drugs. Prior to testing, mice were habituated in the chamber for 30 min daily for three days. All behavioral experiments were carried out with the investigators blinded to treatment conditions. The effects of prolonged administration of these drugs on isoflurane sensitivity were assessed using stepwise increases in isoflurane concentration to establish LORR dose-response curve. LORR test was performed as we previously described [30]. Briefly, mice were exposed to stepwise increased concentrations of isoflurane from 0.4 to 1.4% in 100% oxygen, with an increase of 0.1% every 15 min. The temperature of anesthesia chamber was controlled using DC Temperature Control System (FHC, Bowdoinham, Maine). The rectal temperature of mice during anesthesia was maintained at 37 ± 0.5 °C.

The induction and emergence latencies of anesthetic action were measured using an anesthesia chamber equilibrated with 1.2% isoflurane in 100% oxygen. In mice, 1.2% isoflurane corresponds to ED_{99} for induction [31, 32]. The time taken for a mouse to show loss righting reflex (LORR) was recorded as induction latency. After induction, the mouse was kept anesthetized for 30 min at the same concentration before being transferred to home cage. Emergence latency was recorded as the time taken for a mouse to regain righting reflex in home cage.

For blood pressure measurement, mice were anesthetized with 2% of isoflurane in 70% O_2 and 30% N_2O. The left femoral artery of the mice was cannulated with polyethylene tube (PE-10) in order to measure the mean arterial blood pressure (MABP) with BP transducer. Arterial blood pressure was continuously monitored for 10 min. Blood gases (pCO2, and pO2) and pH were analyzed with a blood gas analyzer (RAPIDLab®, SIEMENS, IL, US). The physiological parameters were measured one day after behavioral testing.

Statistical analysis

Data were analyzed using GraphPad Prism software (version 8 for Windows, GraphPad Software. CA, US) by an investigator blinded to the treatment groups. All of the animals tested were included in the data analysis. LORR curves were fitted by a sigmoidal dose-response model for nonlinear regression [8]. EC50 comparisons were conducted by using GraphPad Prism's compare-option for the unshared parameter EC50 in nonlinear regression. EC50 estimates were expressed as 95% confident intervals (CIs). Induction and emergence latencies were analyzed using t-test. The statistically significant level alpha was set at 0.05.

Results

Epothilone D alters the sensitivity to isoflurane in mice

Sensitivity to isoflurane was analyzed after mice were treated with epothilone D (2 mg/kg, i.p.) [28] daily for two weeks. When the dose of isoflurane was stepwise increased, epothilone D treatment caused leftward shift of the LORR curve (95% CIs for EC50: epothilone D, 0.75 [0.73, 0.77] vs. saline, 0.97 [0.96, 0.98]; $P < 0.01$) (Fig. 1A; epothilone D: $n = 12$, saline: $n = 12$). Compared with saline treatment, epothilone D treatment showed a trend for shorter induction (Fig. 1B; $P = 0.08$) and prolonged emergence latency (Fig. 1C; $P = 0.09$) but did not reach statistical significance at 1.2% isoflurane. Epothilone D treatment did not significantly change blood pressure in mice. The blood pH and CO_2 level was changed in epothilone D treated mice (Table 1). There were no significant differences in body weight between saline treatment (39.6 ± 0.8 g, $n = 12$) and epothilone D treatment (39.6 ± 0.5 g, $n = 12$, $P = 0.25$).

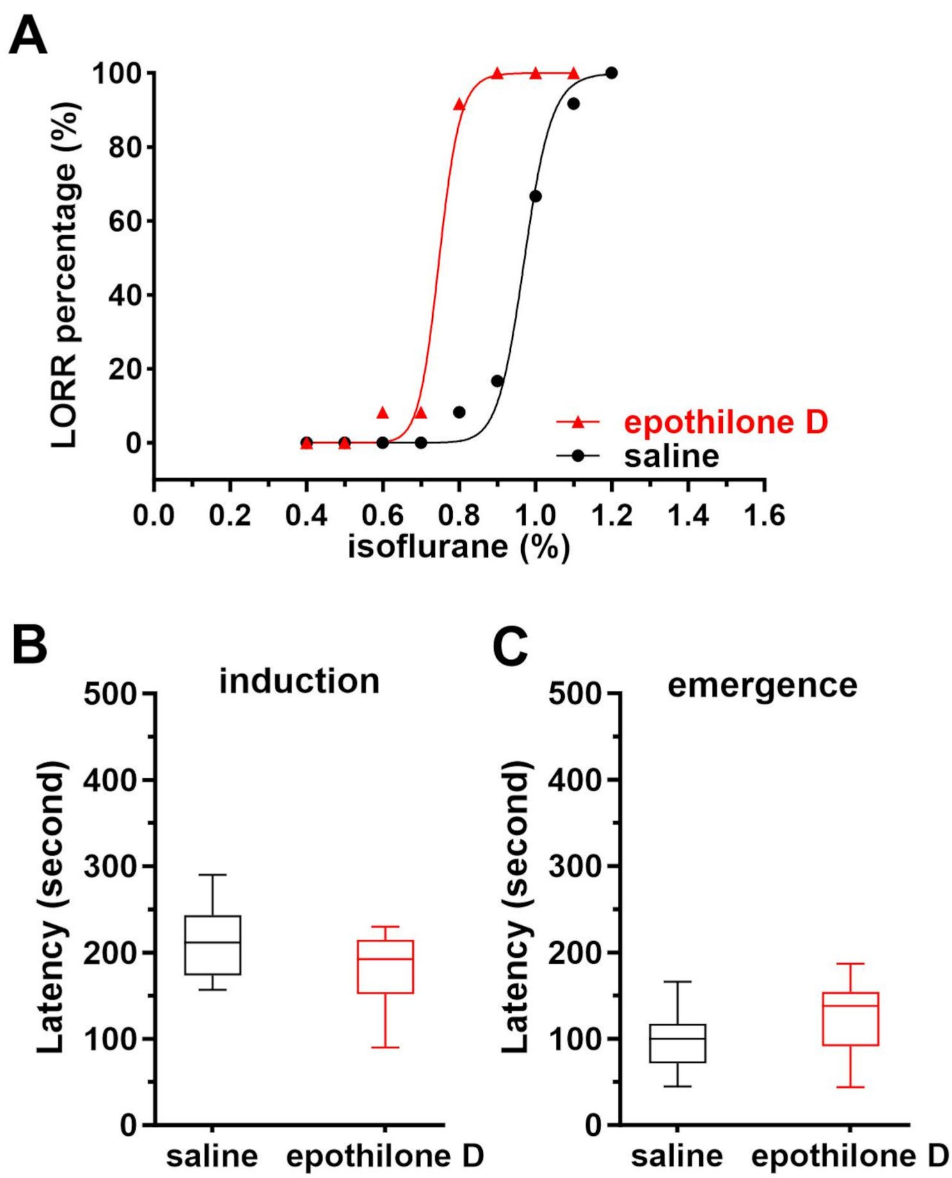

Fig. 1 Epothilone D increased sensitivity to isoflurance in mice. Mice were treated with 2 mg/kg (i.p.) epothilone D or saline daily for 14 day (epothilone D, $n=12$; saline, $n=12$). Behavioral tests were performed at ∼24 h after the drug treatment. (**A**) Epothilone D treatment induced left-ward shift of LORR: EC50: epothilone D, 0.75[0.73, 0.77] vs. saline, 0.97[0.96, 0.98]. (**B**, **C**) At 1.2% isoflurane in 100% oxygen, no significant differences were observed in latency time for induction ($P=0.08$) or emergence ($P=0.09$) between epothilone D and saline treatment groups (Tukey plots)

Table 1 Arterial blood gas (ABG) test and blood pressure measurement of epothilone D and morphine treated mice (mean ± SD, n = 4/group). MABP: mean arterial blood pressure

Treatment	pH	pCO_2 (mmHg)	pO_2 (mmHg)	MABP (mmHg)
saline	7.403 ± 0.03	31.8 ± 5.02	160.1 ± 20.21	91.1 ± 3.74
epothilone D	7.338 ± 0.01	40.5 ± 4.36	163.1 ± 7.70	96.4 ± 11.82
morphine	7.394 ± 0.05	30.5 ± 2.78	169.1 ± 8.82	88.9 ± 11.24

Morphine affects the sensitivity of isoflurane in mice

Mice were treated with morphine (10 mg/kg, i.p.) [29] daily for two weeks. Morphine treatment shifted the LORR curve to the right (morphine, 1.16[1.15, 1.16] vs. saline, 0.97[0.96, 0.98]; $P<0.01$) (Fig. 2A; morphine: $n=11$, saline: $n=12$), increased induction latency at 1.2% isoflurane (Fig. 2B; $P=0.003$) and reduced emergence latency (Fig. 2C; $P=0.02$). Morphine treatment did not significantly change blood pressure, blood gases and pH in mice (Table 1). The mean bodyweight of morphine treatment group (37.3 ± 0.4 g, $n=11$, $P=0.02$) was slightly lower than that of saline treated mice.

Vinblastine increased sensitivity to isoflurane in mice

Vinblastine (0.2 mg/kg, i.p.) was administered daily for five weeks [21]. Compared with saline treatment,

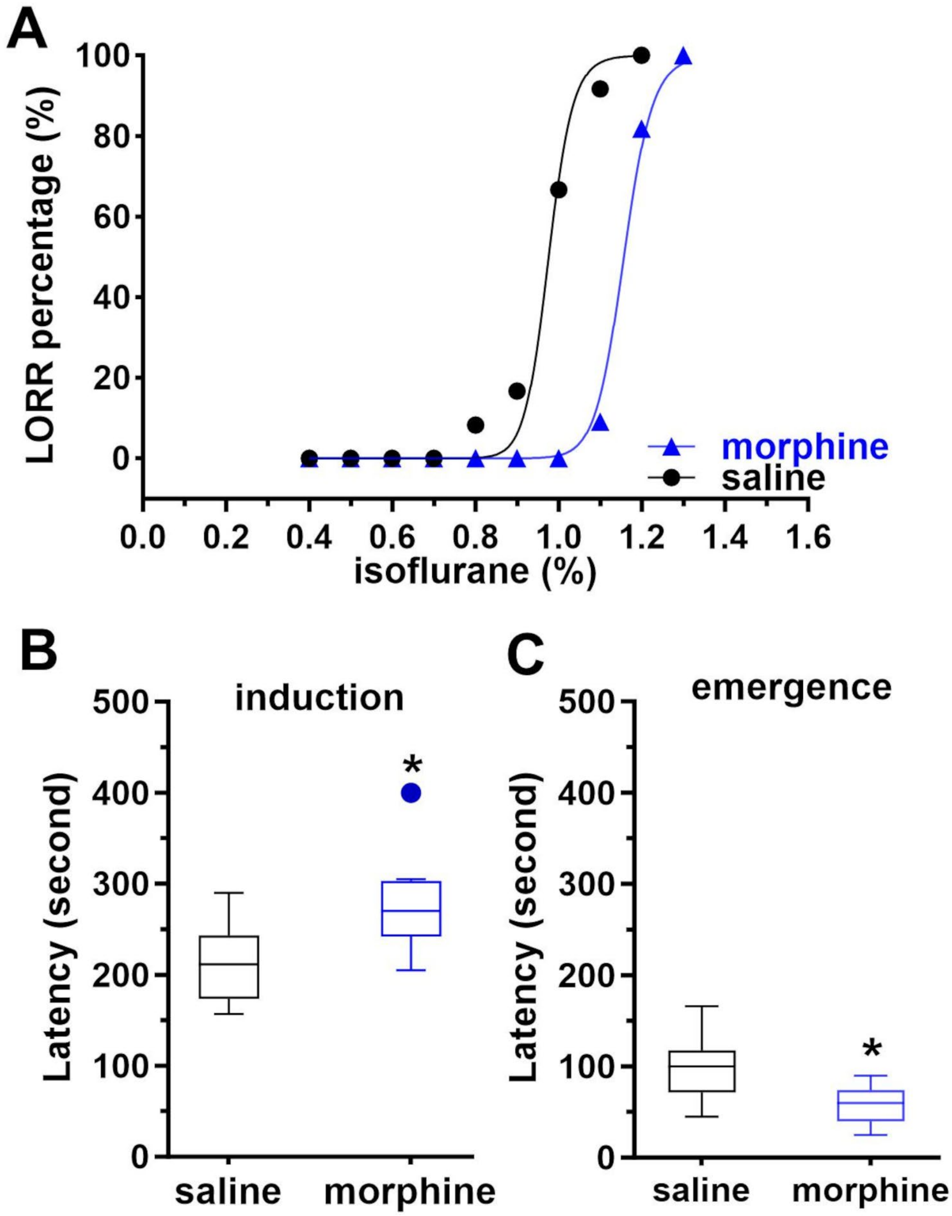

Fig. 2 Morphine caused resistance to the anesthetic effects of isoflurane in mice. Mice were treated with 10 mg/kg (i.p.) morphine daily for 14 day (morphine, $n=11$; saline, $n=12$). Behavioral tests were performed at ~ 24 h after the drug treatment. (**A**) Morphine treatment induced a rightward shift of LORR at stepwise increased isoflurane concentrations: morphine, 1.16[1.15, 1.16] vs. saline, 0.97[0.96, 0.98]. (**B**, **C**) Consistent with rightward shift of induction dose, mice when subjected to 1.2% isoflurane in 100% oxygen, morphine treated mice had extended induction latency ($P=0.003$) and shortened emergence latency ($P=0.02$) compared with saline treated mice (Tukey plots)

vinblastine treatment caused a leftward shift of the LORR response curve (vinblastine, 0.74 [0.73, 0.75] vs. saline, 0.98 [0.97, 0.99]; $P<0.01$) (Fig. 3A; vinblastine: $n=11$, saline: $n=12$). At 1.2% isoflurane, vinblastine treatment decreased both induction latency (Fig. 3B; $P=0.03$) and emergence latency from 30 min of anesthesia (Fig. 3C; $P=0.001$) compared with saline treatment. Vinblastine treatment did not significantly change blood pressure, blood gases or pH in mice (Table 2). There were no significant differences in bodyweight between saline treatment (42.1 ± 1.1 g, $n=12$) and vinblastine treatment (42.2 ± 1.0 g, $n=11$, $P=0.9$).

Paclitaxel affected sensitivity to isoflurane in mice

Paclitaxel (20 mg/kg, i.p.) was administrated three-doses per week for four weeks; [20] Paclitaxel treatment marginally shifted the LORR response curve to the right at stepwise increased isoflurane concentrations (paclitaxel, 1.05[1.03, 1.06] vs. saline, 0.98 [0.97, 0.99]; $P<0.01$) (Fig. 4A; paclitaxel: $n=11$, saline: $n=12$).

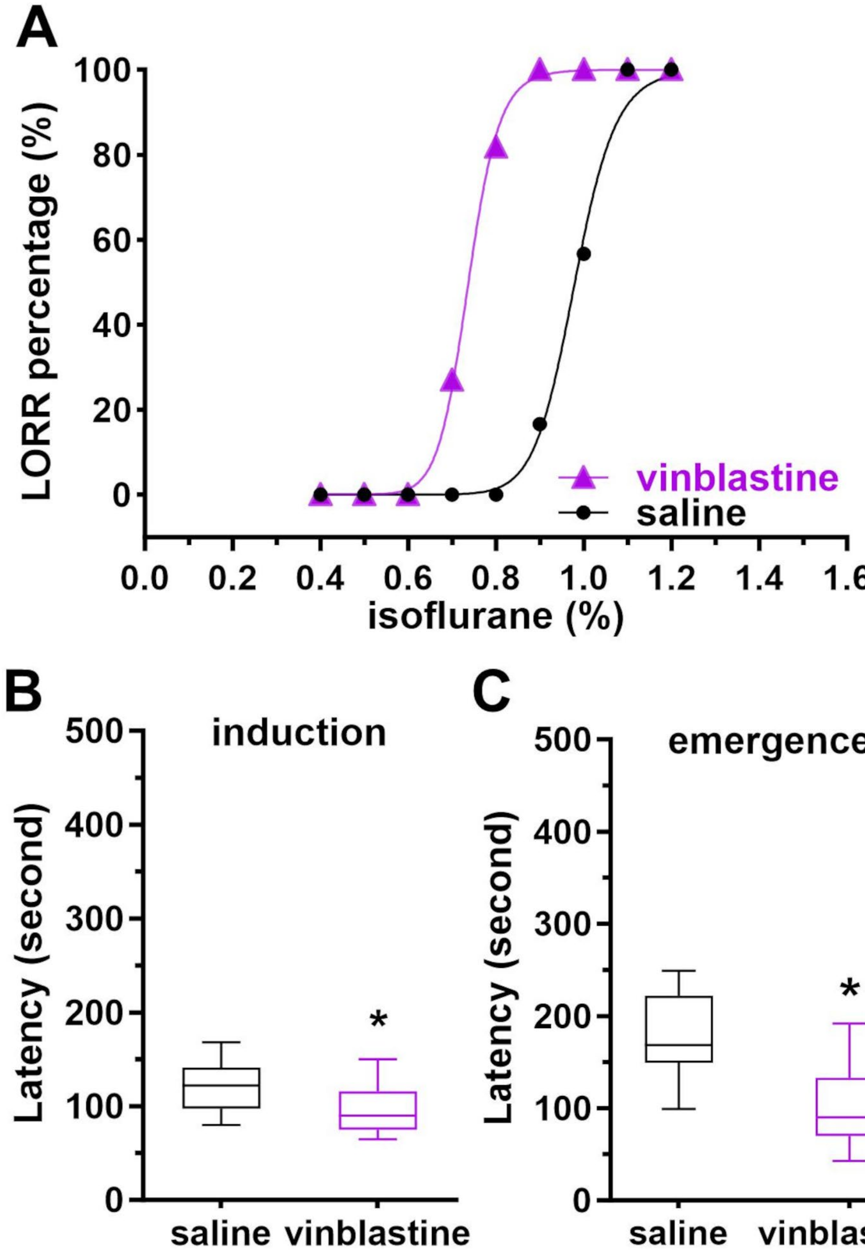

Fig. 3 Vinblastine treatment increased sensitivity to isoflurane in mice. Mice were treated with 0.2 mg/kg (i.p.) vinblastine or saline daily for 5 weeks (vinblastine, $n=11$; saline, $n=12$). Behavioral tests were performed at ∼24 h after end of drug treatment. (**A**) Vinblastine treatment induced a leftward shift of LORR: vinblastine, 0.74[0.73, 0.75] vs. saline, 0.98[0.97, 0.99]. (**B**, **C**) At 1.2% isoflurane in 100% oxygen, both induction and emergence latencies were decreased in vinblastine ($P=0.03$ and $P=0.001$ respectively) compared with saline treatment in mice (Tukey plots). The discrepancy between induction and emergence with isoflurane is possibly related the hysteresis between washin and wahout of the anesthetic [52]

Table 2 Arterial blood gas (ABG) test and blood pressure measurement of paclitaxel and vinblastine treated mice (mean ± SD, n = 4/group). MABP: mean arterial blood pressure

treatment	pH	pCO_2 (mmHg)	pO_2 (mmHg)	MABP (mmHg)
saline	7.390 ± 0.05	34.2 ± 8.52	160.8 ± 12.78	83.4 ± 10.71
paclitaxel	7.386 ± 0.04	32.4 ± 6.75	166.6 ± 14.75	88.2 ± 9.36
vinblastine	7.342 ± 0.04	32.7 ± 5.36	156.7 ± 8.52	78.0 ± 4.21

At 1.2% isoflurane, paclitaxel and saline treated groups did not significantly differ in induction (Fig. 4B; $P=0.5$) or emergency (Fig. 4C; $P=0.3$) latency. Paclitaxel treatment did not significantly change blood pressure, blood gases or pH in mice (Table 2). The paclitaxel treatment group (36.5 ± 0.1.8 g, $n=11$, $P=0.01$) had a lower mean bodyweight than saline treated mice. Taken together, paclitaxel treatment only slightly affected sensitivity to isoflurane in mice.

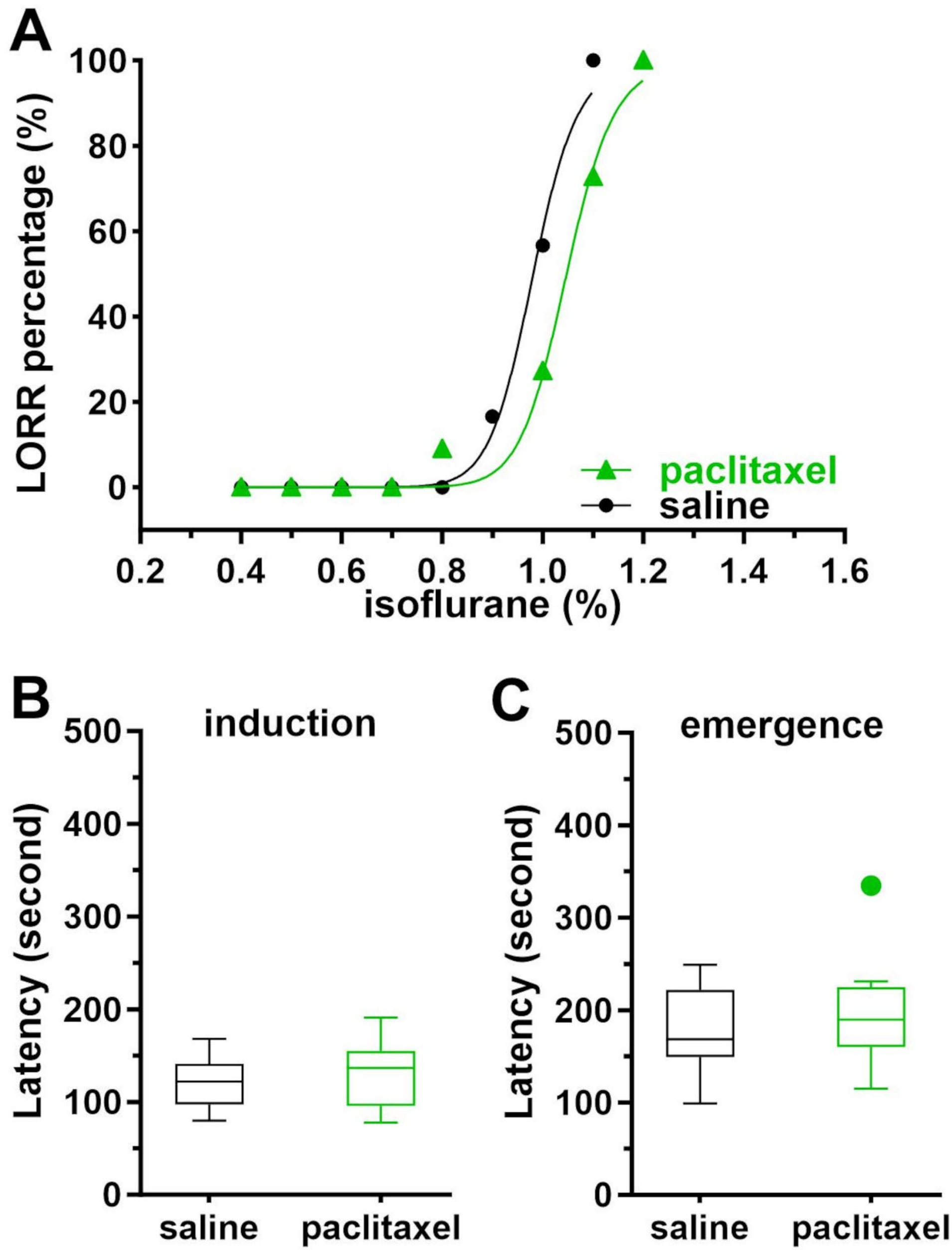

Fig. 4 Paclitaxel treatment shifted isoflurane sensitivity to the right in mice. Mice were treated with 20 mg/kg (i.p.) paclitaxel 3 times a week for four weeks (paclitaxel, $n=11$; saline, $n=12$). Behavioral tests were performed at ∼24 h after end of drug treatment. (**A**) Paclitaxel treatment marginally but significantly right shifted the LORR curve in mice: paclitaxel, 1.05[1.03, 1.06] vs. saline, 0.98 [0.97, 0.99]. (**B**, **C**) At 1.2% isoflurane in 100% oxygen, both induction and emergence latencies were comparable between paclitaxel and saline groups ($P=0.58$ and $P=0.34$) (Tukey plots)

Discussion

Our study highlights a possible role of MTDs in modulating anesthetic effects in disparate directions, which has implications for anesthetic concentrations that should be used for induction, maintenance, and emergence of anesthesia. The findings in rodents may have relevance to the perioperative care of cancer patients who receive MT-targeting chemotherapy drugs or even opioids for pain for prolonged periods. This study also provide additional experimental evidence to support the hypothesis that microtubles is a functional target of anesthetics [13, 33].

Anesthetics cause loss of consciousness by acting on one or more moleculars including membrane receptors and ion channel proteins [34]. It has been postulated that MTs of neurons are one of the molecular targets for anesthetic-induced loss of consciousness [33], supported by experimental [7] and computer-modeling investigations [12, 35]. Moreover, both in vivo [36] and in vitro [9, 37] studies have shown that anesthetics affect the assembly of microtubules, alter microtubule dynamics and stability in cells. We tested the hypothesis that MTDs affect the anesthetic action of isoflurane. Our data demonstrated that drugs with microtubule modulating activity, specifically chemotherapeutic drugs, epothilone D and vinblastine, shifted the dose-response curves (ED50) of isoflurane to the left in mice. Paclitaxel treated mice were slightly resistant to isoflurane. MTDs interact with tubulins/microtubules via distinct sites/mechanisms of action, which may explain the different responses. Epothilone D and paclitaxel are MT stabilizers and they promote MT polymerization by binding to β-tubulin subunits in distinct manners [38]. Epothilone D also has a higher affinity for β-tubulin subunits than paclitaxel [39]. Vinblastine destabilizes MTs by interacting with αβ-tubulin heterodimer to inhibit polymerization of MTs [40]. MTDs increase the accumulation of tubulin post-translational modifications (PTMs), such as acetylation, detyrosination, tyrosination, and detyrosination, affecting microbutule dynamics. However, the functional relationship between PTMs and the binding of anesthetics, as well as the relationship between PTMs and microtubule dynamics, is not fully understood.

We have only examined three drugs that are of significance in the discovery of MTDs for clinical application and are still being used in cancer therapy. Vinblastine is the first FDA-approved anticancer chemotherapy drug and has been used over 50 years [41, 42]. Paclitaxel is the first identified MT stabilizer [43] and was approved by the FDA in 1990s. Epothilones are a class of recently identified MT stabilizers with anti-tumor activities, among which only epothilone D can cross the blood-brain barrier [44] and has been explored for its protective effects in neurodegenerative disease [45] and traumatic injury to central nerve systems [28]. Both our data and a recent study [46] show that MTDs significantly affect the action of isoflurane in inducing unconsciousness, suggesting that more clinical studies are needed to examine how MTD chemotherapy drugs affect cancer patients' reaction to anesthetics. As MTDs remain among the most effective anticancer agents [17], other important MTDs should be investigated for their impact on anesthetic action.

Morphine affects the expression and post translational modification of α-tubulin and microtubule-associated proteins [23, 47, 48]. Microtubule-associated proteins, such as Tau and stathmin, are proteins that bind to tubulin subunits to regulate microtubule stability [49]. In rats, chronic morphine treatment changes the expression levels of α-tubulin, Tau, and stathmin in the striatum [23]. In microglia EOC13 cells, morphine treatment decreased acetyl-α-tubulin levels [47]. Moreover, morphine not only has specific actions on the opioid receptors but also modulates GABAergic system [50], Similarly, general anesthetics interact with both opioid [51] and GABA receptors [5]. Therefore, morphine induced changes in isoflurane sensitivity could be multifactorial. Our data indicate that morpine induces resistance to the effects of isoflurane evidenced by the rightward shift of the dose-response curve, longer induction and faster wake times.

We demonstrated that microtubule modulating drugs affect sensitivity to isoflurane in mice. However, this study has its limitations. Some of the chemotherapeutic drugs have poor water solubity, such as paclitaxel and epothilone D examined in this study. Although these drugs were further diluted from the stock solution in saline for treatment, more proper control for the vehicle other than saline would have been a better choice. Minimum alveolar concentration (MAC), as the standard measure of potency for inhaled anesthetics in human studies, was not used in this study; we only used LORR, a common measure for rodent studies. In fact, some studies have expressed the concerns regarding the use of MAC as measure of potency of anesthetics because of hysteresis that is seen with induction and emergence of anesthetics [52]. Measurement of MAC values in mice could help us better understand the effects of microtubule-modulating drugs on the potency of inhaled anesthetics. For LORR and RORR experiments, the saline groups in two cohorts exhibited a large difference in latency (not a MAC value), which could be attributed to the following: the experiments conducted on these two cohorts of mice (which were purchased separately) about ~ three months apart and the CD1 mice were outbred. The advantage of using outbred mice is that they better represent the genetic diversity seen in human populations, and a study has found that CD1 mice have variation in sensitivity to isoflurane [53]. Moreover, only isoflurane was evaluated in this study. Other anesthetics, such as sevoflurane and desflurane, are also commonly used, and examining the impacts of microtubule-modulating drugs on sensitivity to these anesthetics will provide a better understanding of the mechanisms involved. Patients' age [54, 55] and sex [56] affect their responses to general anesthesia and recovery. Age- and sex-related differences in brain microtubules and microtubule associated proteins have been observed in mice [57, 58]. Therefore, mice of both sexes of different ages should be included in future studies. In this study, we report that MTDs affect the sensitivity to isoflurane in mice. Further studies are warranted to establish a causal relationship between microbutule dynamics and the anesthetic effects when tools become available to examine microtubule dynamics in vivo.

In summary, our data indicate that chemotherapeutic drugs with microtubule-modulating activities affect sensitivity to isoflurane in mice. The data emphasize the need for more pre-clinical and clinical studies on the link between microtubule modulating drugs and sensitivity to general anesthesia. These studies will be especially beneficial to cancer patients receiving MTD neoadjuvant therapy, and patients on opioid therapy or with opioid use disorders. Our work has set the stage for future studies of cellular and molecular mechanisms of brain microtubules in general anesthesia.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12871-025-02956-9.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

The authors are grateful to Scot Mackeil (Bioengineer Lab, MGH) for his expertise in anesthesia equipment maintenance and calibration.

Author contributions

Z.Y., J. M., and JAJ. M. conceived the project and wrote the manuscript. N. L., Z. Y., and Y. R. performed the behavioral experiments. H. K. and G. L. measured blood pressure and analyzed blood gases and pH. J. Y. performed statistical analysis and helped with the manuscript preparation. J.T.D., W.D., S. X., S. W., X Z., X.W., S.S., Y. D., and Z.X. helped with behavioral study. L. C. helped with the manuscript preparation.

Funding

The work is partially supported by grants from NIH R01 GM118947 and R01 GM142042 and Shriners Hospital Research Philanthropy (to JAJM) and NIH R01 DA36564 and Richard J. Kitz Endowed Professorship of Harvard Medical School (to JM).

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

The study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org). Animal protocol (2017N000233) was approved by MGH IACUC.

Consent for publication

Not applicable, no human subject is involved.

Competing interests

The authors declare no competing interests.

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Received: 17 January 2024 / Accepted: 9 February 2025
Published online: 28 February 2025

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