THE EFFECTS OF VALERIAN ON THE TIME COURSE OF EMERGENCE FROM GENERAL ANESTHESIA IN SPRAGUE-DAWLEY RATS (RATTUS NORVEGICUS)

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Herbal use may be associated with increased morbidity and mortality as a consequence of interactions with anesthetic agents. The purpose of this study was to investigate the effects on emergence from isoflurane anesthesia using a combination of the herb valerian and midazolam compared with valerian alone, midazolam alone, and no additional drug-herb treatment in Sprague-Dawley rats.

We assigned 32 male Sprague-Dawley rats to 1 of 4 groups: (1) isoflurane alone, (2) isoflurane plus valerian, (3) isoflurane plus midazolam, and (4) isoflurane plus a combination of valerian and midazolam. Thirty minutes after treatment, animals underwent a standard laparotomy. The time in seconds from discontinuation of isoflurane to the time the animal righted itself and took 1 step was recorded as emergence time.

A 1-way analysis of variance with a post hoc Scheffe procedure revealed that animals given a combination of midazolam and valerian took significantly longer to emerge from anesthesia ($F = 58.21; P < .00$) compared with all other groups.

Awareness of possible interactions of herbals with conventional anesthetics is important so that potential problems may be recognized and treated. These data demonstrate the need for continued research concerning the effects of herbals and their potential for interaction with anesthetics.

Key words: Benzodiazepines, emergence, rats, valerian.

Nearly 25% of all patients admitted for surgery take some type of herbal preparation. Patients most commonly report using herbals for chronic back problems, headache, anxiety, depression, or insomnia. However, herbal use may be associated with increased morbidity and mortality as a consequence of interactions with anesthetic agents or herbal-induced alterations of physiologic functions.

To alleviate insomnia, many Americans take herbal medications such as valerian. Valerian may be used as an anxiolytic or as a treatment for insomnia. Historically, valerian was used for medicinal purposes by the Greeks, Romans, and Chinese. In fact, Hippocrates, Galen, and Culpeper, all early herbalists, noted the sedative and anxiolytic properties of valerian. Sesquiterpines, volatile organic compounds found in valerian, and $\gamma$-aminobutyric acid (GABA), the inhibitory neurotransmitter in the central nervous system, also found in valerian, are believed to be responsible for most biological effects of valerian by inhibiting the breakdown or enhancing the effect of GABA in the brain, producing sedation. No study to date has evaluated the effects of valerian or the combination of valerian and midazolam, a commonly used preanesthetic anxiolytic, on emergence time from general anesthesia in animals or humans.

The popularity of herbal medicines such as valerian may have substantial implications for healthcare professionals, specifically anesthesia providers. For example, only about 40% of patients inform their physicians of their use of alternative medicines. Furthermore, more than 15 million people take prescription medications concurrently with herbal medications and/or high-dose vitamins. This may present substantial potential for adverse interactions between anesthetics and herbal medications.

The purpose of this study was to investigate the
effects of valerian on emergence from isoflurane anesthesia using a combination of valerian and midazolam compared with valerian alone, midazolam alone, and no additional drug-herb treatment in rodents. Two research questions directed this study. First, does valerian prolong emergence from general anesthesia in rodents? Second, does the combination of valerian and midazolam prolong emergence from general anesthesia compared with midazolam alone in rodents?

Materials and methods
Thirty-two mature male Sprague-Dawley rats, weighing 225 to 250 g were used in this experiment with 4 additional animals used for technique development. The study was approved by the 59th Medical Wing Clinical Research Squadron Institutional Animal Care and Use Committee located at Lackland AFB, Texas. Animals were acclimatized to a vivarium for 24 hours before the experiment. All were housed separately in cages. Animals had continuous access to food and water except for the 8 hours before the experiment, when only water was available.

The rats were divided into the following 4 groups: (1) isoflurane alone, (2) isoflurane plus 30 mg/kg of valerian (Jamieson Laboratories, Toronto, Ontario, Canada) administered by gavage due to the composition of the compound, (3) isoflurane plus 2 mg/kg of midazolam administered via intramuscular injection, and (4) isoflurane plus a combination of 30 mg/kg of valerian and 2 mg/kg of midazolam. Jamieson Laboratories provided a high-performance liquid chromatography assay of the standardized product from which we based the dosing of valerian. Thirty minutes before the experiment, animals in groups 2 and 4 were administered valerian (30 mg/kg) by oral gavage. Fifteen minutes before the experiment, animals in groups 3 and 4 were administered midazolam (2 mg/kg) via intramuscular injection.

A round of surgery was performed including 1 rat from each group, for a total of 4 rats per round, to control for the potential confounding effects of circadian rhythm. In this study, a round of surgery was defined as a standard laparotomy with isoflurane anesthesia. All rats were induced with 5% isoflurane in separate induction chambers of a rodent anesthesia machine. After induction, the rats were removed from the chamber and placed on the operating table with snouts firmly placed in anesthesia delivery cones at 2% isoflurane. The abdomen was then shaved and prepared with povidone iodine.

Surgery consisted of a 4-cm midline incision through the skin and abdominal muscle wall followed by the externalization of a 10-cm segment of the small intestine for a period of 4 minutes. During the first minute of externalization of the bowel, the intestine was gently rubbed between 2 pieces of gauze in 4 locations as a standard irritant; this procedure promotes the release of local inflammatory mediators. For the remainder of the 4-minute period, the intestines were covered with saline-soaked gauze to maintain the moisture content. The intestines were then returned to the abdominal cavity and irrigated with saline and the muscle and skin layers sutured with 5/0 monofilament wire.

Isoflurane anesthesia was continued for 2 additional minutes following surgery. After this 2-minute period, the isoflurane was turned off, and a stopwatch was started. The rats were observed until the righting reflex was completed. For this experiment, emergence time from anesthesia was defined as the total time, in seconds, from the time the vaporizer was turned off until the time the animal righted itself and took 1 step. Immediately after the experiment, all animals were killed humanely.

Results
The analysis of variance comparing emergence times

![Figure. Emergence time in seconds among the groups](image-url)
among groups revealed a significant overall difference
\( (F = 58.21; \ P < .00) \) with a post hoc Scheffe analysis
suggesting the following. As expected, rats in group 3
had a significantly prolonged emergence time from
general anesthesia (mean time, 326 seconds) com-
pared with rats in group 1 (mean time, 123 seconds; \( P < .00 \)). Rats in group 3 also had a significantly pro-
longed emergence time compared with rats in group 2
(mean time, 127 seconds; \( P < .00 \)). The most interest-
ning finding was that rats in group 4 had a significantly
prolonged emergence time from general anesthesia
(mean time, 452 seconds) compared with all other
groups, including those in group 3, suggesting an
additive or synergistic effect of valerian when com-
bined with the commonly used preanesthetic anxi-
olytic, midazolam (\( P < .03 \)). This is graphically repre-
sented in the Figure.

Two animals from group 4 were excluded from data
analysis due to extremely prolonged emergence times
(1,075 and 1,545 seconds). These 2 emergence times
were twice as long as any animal in any group.
Whereas including those data would have strength-
ened the suggestion that valerian is additive or syner-
gistic with midazolam, it is well known that including
extreme outliers in analysis of data for small samples
may misleadingly influence the magnitude of signifi-
cance.\(^6\) Therefore, so as not to report misleading sta-
tistics, final analysis included data from the remaining
6 rats in group 4 plus the data from the 8 rats in all
other groups. These data met the assumption of
homogeneity of variance as measured by the Levene
test (\( P = .093 \)). The Table summarizes the statistical
data for each of the groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cases</th>
<th>Mean emergence comparison time (s)</th>
<th>Range (s)</th>
<th>SD</th>
<th>Post hoc ( P ) values</th>
</tr>
</thead>
</table>
| 1, Isoflurane alone                         | 8            | 123.50                            | 95.0-179.0| 29.02| Compared with group 2, \( P = .99 \)
|                                            |              |                                   |           |     | Compared with group 3, \( P = .00 \)
|                                            |              |                                   |           |     | Compared with group 4, \( P = .00 \)
| 2, Isoflurane and valerian                  | 8            | 127.38                            | 60.0-179.0| 41.85| Compared with group 1, \( P = .99 \)
|                                            |              |                                   |           |     | Compared with group 3, \( P = .00 \)
|                                            |              |                                   |           |     | Compared with group 4, \( P = .00 \)
| 3, Isoflurane and midazolam                 | 8            | 326.88                            | 243.0-360.0| 38.84| Compared with group 1, \( P = .00 \)
|                                            |              |                                   |           |     | Compared with group 2, \( P = .00 \)
|                                            |              |                                   |           |     | Compared with group 4, \( P = .03 \)
| 4, Isoflurane and the combination of        | 6            | 452.50                            | 311.0-615.0| 101.78| Compared with group 1, \( P = .00 \)
| valerian plus midazolam                     |              |                                   |           |     | Compared with group 2, \( P = .00 \)
|                                            |              |                                   |           |     | Compared with group 3, \( P = .03 \)

Table. Descriptive and inferential statistics of groups with sample size, mean time to emergence, range, SD, and Scheffe post hoc comparisons

Discussion

The chemical composition of valerian includes
esesquiterpines of the volatile oils (valeric acid and iso-
valeric acid), ketones (valeranone), terpinoid esters
(valepotriates) and their decomposition products
(baldrinals), alkaloids, lignans, and free amino acids
such as GABA, tyrosine, arginine, and glutamine. The
baldrinals have been shown to reduce spontaneous
motility in mice, but sesquiterpines of the volatile oils
and GABA are believed to be responsible for most bio-
logical effects of valerian by inhibiting the breakdown
or enhancing the effect of GABA in the brain, produc-
ing sedation.\(^2\) Marder et al\(^7\) isolated 6-methylapigenin,
a flavone derivative, that is a ligand for the benzodi-
azepine-binding site of the GABA\(_A\) receptor and her-
peridin, a flavanone glycoside with sedative and sleep-
enhancing properties from several different varieties
of the Valeriana genus. Most studies suggest that the
sedation and anxiolytic properties of valerian are a
synergistic effect from the several constituents of the
valerian root.

The limited number of studies available in the lit-
erature examining valerian and sleep time (or, con-
versely, emergence time) using a rodent model have
suggested that valerian prolongs emergence time in
the presence of barbiturates. For example, Sakamoto
and colleagues\(^8\) compared the effects of valerian (11.2
g/kg) and diazepam (3 mg/kg) on sleep time in ddY
mice when administered in conjunction with hexo-
obarbital (100 mg/kg) with hexobarbital alone. Valer-
ian or diazepam was administered by oral gavage 30
minutes before an intraperitoneal injection of hexo-
obarbital. The time required for the mice to exhibit the
righting reflex (ability to stand on their feet) was used
to define the end of sleep. The investigators reported that valerian and diazepam significantly prolonged hexobarbital-induced sleep compared with hexobarbital alone. 8

Valerian was also found to prolong sleep time in NMRI mice when combined with thiopental, suggesting that valerian has central nervous system depressant activity. 9 By using the righting reflex as the definition of wakefulness, researchers compared the effects of the administration of valerian plus thiopental with thiopental plus chlorpromazine or thiopental alone. The results suggested that valerian increased sleep time in a dose-dependent manner. Sleep time was increased by a factor of 1.6 in mice given 2 mg/kg of valerian compared with mice given thiopental alone and 7.6 times in mice given 200 mg/kg of valerian. The administration of chlorpromazine increased sleep time 4.7-fold compared with administration of thiopental alone.

In the present study, whereas rats given isoflurane and valerian did not exhibit prolonged emergence compared with rats given isoflurane alone, animals given isoflurane plus a combination of valerian and midazolam had a significantly prolonged emergence from isoflurane anesthesia compared with animals that received isoflurane alone or isoflurane and midazolam. Midazolam, a benzodiazepine, has a molecular mechanism of action similar to the barbiturates such that it binds to the GABA A receptor. 10 Similar to the findings from studies comparing sleep time (or emergence time) when rodents are administered a combination of valerian and a barbiturate, 8,9 the findings from this study suggest that valerian produces an additive or synergistic effect when administered with midazolam.

There was no significant difference in emergence time between rats given isoflurane and valerian compared with rats given isoflurane alone. This paradoxical finding may suggest that the effects of valerian at the dose used in this study are dependent on the presence of an anesthetic agent that is a specific and long-acting GABA A receptor agonist (midazolam or the barbiturates) rather than an inhalation anesthetic (isoflurane) that is purported to have GABA A activity but for which the complete mechanism of action remains unclear. Furthermore, the half-life and metabolism of isoflurane are considerably different from those of midazolam and the barbiturates. The elimination of isoflurane depends on the blood-gas solubility and length of administration. Isoflurane was administered for approximately 15 to 20 minutes in this study; therefore, there was very little accumulation in the tissue contributing to prolonged emergence. The elimination half-life of isoflurane after a short duration of administration is rapid, whereas the elimination half-lives of midazolam and thiopental (a barbiturate) are 1 to 4 hours and 11.6 hours, respectively. 10 Moreover, inhaled isoflurane is predominantly eliminated unchanged via the lungs, with only approximately 0.2% metabolized by cytochrome P-450 enzymes in the liver. In contrast, midazolam and barbiturates are extensively metabolized by the liver.

Taken together, we speculate that the effects of valerian, when administered with longer acting GABA A agonists such as midazolam, may be more potent and more prolonged than when administered with a shorter acting GABA A agonist such as isoflurane. Thus, rodents that received valerian in combination with midazolam had a significantly longer emergence time than those that received midazolam alone. Alternatively, the finding of no significant difference in emergence time between rats given isoflurane and valerian compared with rats given isoflurane alone may have been a consequence of the method of administration of valerian compared with midazolam. Valerian is a large, complex composite of volatile oils, other hydrocarbons, and free amino acids. The valerian product purchased from Jamieson Laboratories, as such, only completely solubilized in percentages of organic solvents such as dimethylsulfoxide or alcohol in saline or water that would have resulted in neurotoxic effects. Therefore valerian was administered as a suspension in sterile water via gavage. Absorption of valerian from the gastrointestinal tract may not have been as complete or as rapid as anticipated, despite allowing 30 minutes for absorption, compared with the absorption of midazolam via the intramuscular route of administration.

Finally, the single 30-mg/kg oral dose of valerian administered preoperatively had no significant effect on emergence time compared with no drug treatment. This finding may have been a result of the chosen dose, the route of administration, the 1-time administration, or a combination of these variables. Research in human studies suggests that the effective oral dose of valerian to achieve anxiolytic and hypnotic effects ranges from 400 to 900 mg administered daily. 3,11 Furthermore, long-term administration of valerian for a minimum of 2 weeks may be necessary to achieve pharmacologically effective serum levels. 12

The use of alternative medicine is prevalent in the United States. 5 Of the US population, 42% acknowledges trying at least 1 form of alternative medicine such as valerian as a sleep aid. The findings of studies...
in humans are conflicting regarding the efficacy of valerian. For example, Leathwood et al13 found that a 400-mg aqueous extract of valerian extract significantly decreased sleep latency, defined as time to fall asleep, and improved sleep quality compared with placebo or a commercially available valerian-hops preparation. The findings from this study suggest that (1) a single dose of the active constituents of valerian extract may have efficacy, and (2) the active constituents of valerian may be more efficacious than the commercially available product sold over the counter.

In contrast, a randomized, placebo-controlled, double-blind, crossover study demonstrated by polysomnography that a single 600-mg dose of the valerian extract had no effects on sleep efficiency. However, a multiple dose regimen for 14 days resulted in significant improvement in slow-wave sleep.14

**Conclusion**

To our knowledge, this is the first study to compare the effects of valerian or the combination of valerian and midazolam, a commonly used preanesthetic anxiolytic, on the emergence time from general anesthesia in animals or humans. These findings suggest that the combination of valerian and midazolam may prolong emergence time. Nevertheless, caution should be used when generalizing findings from animal studies to humans. More studies are needed to corroborate our findings, and we recommend clinical trials to evaluate the effect of valerian on emergence time in humans.

**REFERENCES**


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