# Co-administration of JM-1232(-) reduces the dose of propofol required for hypnosis with minimal prolongation of recovery time even after repeated injections

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**Research Article**

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# Abstract

**Background:** Drug interaction is an important and effective phenomenon in the area of anesthesiology because of drugs combinations potentially possessing novel properties. The characteristics of anesthesia with co-administration of JM-1232(-), benzodiazepine receptor agonist, and propofol, the most popular anesthetics, was studied using mice.

**Methods:** Male adult mice were each administered JM-1232(-), propofol and various combinations of the two drugs intravenously. Loss of the righting reflex was evaluated as achieving hypnosis and the time until recovery of the reflex was measured as hypnosis time. After determining the ED50, double and triple the ED50 dose of propofol with JM-1232(-) were injected to compare hypnosis time. The injections were repeated 4 times and each hypnosis time was compared. Separately, flumazenil was administered immediately after the last dose was injected.

**Results:** The ED50s ([95% confidence interval]) for hypnosis were 3.76 [3.36 - 4.10] for JM-1232(-) and 9.88 [8.03 - 11.58] mg kg-1 for propofol. Co-administration of 0.05- and 0.1-mg kg-1 of JM-1232(-) reduced the ED50 of propofol to 1.76 [1.21 - 2.51] and 1.00 [0.46 - 1.86] mg kg-1. The interaction of the drug combinations for hypnosis was supra-additive. Hypnosis time was significantly shorter in the groups given the mixtures as compared to each hypnotic administered alone. After repeated injections, hypnosis time with the mixtures showed smaller prolongation than that with sole hypnotics. Flumazenil antagonized the supra-additive effects of the mixtures.

**Conclusions:** The combination of JM-1232(-) and propofol shows supra-additive interaction and the reduced hypnotic dose translates to faster recovery even after multiple injections.



**Keywords:** JM-1232(-), Propofol, Supra-additive interaction, Flumazenil.


# Background

JM-1232(-) is a newly developed isoindoline derivative and potential anesthetic, which has a short duration of action [1, 2]. JM-1232(-) is water soluble and is a highly potent substance. Although, the molecular structure of JM-1232(-) is different from classical and typical benzodiazepines, the agent was reported that JM-1232(-) enhanced synaptic inhibition through the modulation of benzodiazepine binding sites on γ-aminobutyric acid A receptors, similar to benzodiazepine derivatives [3]. The pharmacological parameters of JM-1232(-) might be suitable for a supporting drug of general anesthesia and intensive care medicine [4]. Moreover, JM-1232(-) was administered to humans as “MR04A3”, which demonstrated favorable and acceptable profiles in a preclinical trial [5].

Nowadays, propofol is one of the most popular intravenous anesthetics in daily clinical practice [6]. The characteristics of propofol, including rapid onset and prompt recovery, make it an appropriate drug for general anesthesia. However, the long-lasting infusion of propofol might lead to prolongation of its effect and delay in recovery [7]. The dose of propofol required for the induction of anesthesia can be reduced by a

series of pre-medication [8, 9]. Some of these drugs such as benzodiazepine derivatives significantly enhance the hypnotic activity of propofol [10, 11]. Thus, it is possible that co-administration of JM-1232(-) could reduce the required dose of propofol. Moreover, the reduction of propofol might lead to faster recovery.

In the current study, an *in vivo* investigation using mice, the interaction between JM-1232(-) and propofol was evaluated at first. Thereafter, the anesthesia and recovery profiles after repeated injections of the drug mixtures, which simulated prolonged infusion, were investigated. Finally, the animals were administered flumazenil to assess its antagonistic effects after long-lasting anesthesia.

# Methods

After obtaining approval from the Ethics Committee of Animal Experiments at our institution (Final registration number: 26401), all experiments were performed within the animal laboratory. Male adult Deutsch-Denken-Yoken (ddY, closed colony) mice weighing 38 to 45 g (purchased from the company named SLC Japan, Nagoya, Japan) were used. The animals were maintained at a 12/12-h light-dark cycle and fed *ad libitum* before the experiments. All experiments were conducted between 10 a.m. and 4 p.m. The mice were examined three times at most and had a recovery period of more than 7 days.

The mice were set in a transparent animal holder to place a 24G plastic IV cannula (SurFlo, Terumo, Tokyo, Japan) into the tail vein. After confirming venous catheterization by checking the backflow of blood, another customized injection needle connected to a micro-syringe was set into the plastic cannula, and the prepared material was quickly injected over 2-3 s. If the injection was irregular and incomplete, for example, the injection having the resistance or showing the extravasation, the experiment was omitted from the study. Mice were released from the animal holder and individually evaluated for hypnosis on a flat table by another observer. The criterion for hypnosis was loss of the righting reflex, occurring < 10 s after the start of the injection [12, 13]. When hypnosis was observed, the mice were gently placed in the lateral decubitus position until spontaneous recovery to the upright position, which was defined as the end of hypnosis. The time from the start of drug injection to return to the end of hypnosis was defined as hypnosis time. Hypnosis time was recorded at the laboratory room and was verified by another blinded technical assistant staff using recorded movies of experiments in the other day. The animals were killed by inhalation of carbon dioxide after the final experiments.

Propofol (Diprivan, AstraZeneca K.K., Osaka, Japan) was diluted with 10% soybean oil (Intralipid, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). JM-1232(-) was given as a gift from Maruishi Pharmaceutical (Osaka, Japan). The agents were dissolved in physiological saline.

All solutions were mixed with the same volume of diluent and administered intravenously. Injection volume was set at 10 ml $kg^{-1}$ in experiment 1, and at 5 ml $kg^{-1}$ in experiments 2 and 3. The experimental doses of JM-1232(-), propofol and the drug mixtures was calculated by the results of past and preliminary experiments [12, 13] (Table). The $ED_{50}$ of propofol was firstly tested and other combinations

were tried until all of animals in the group showing same responses (achieving or not achieving hypnosis).

Experiment 1: Interaction between JM-1232(-) and propofol

Mice were given either JM-1232(-) (3, 3.5, 4, 4.5 and 5 mg $kg^{-1}$) or propofol (5, 7.5, 10, 12.5 and 15 mg $kg^{-1}$) intravenously to determine the hypnotic effects of various doses of the drugs. Each group was consisted of 6 animals. To evaluate the effect of the combination of JM-1232(-) and propofol, other mice were simultaneously administered JM-1232(-) (0.5, 1 and 2 mg $kg^{-1}$) and propofol (0.625, 1.25, 2.5, 3.75 and 5 mg $kg^{-1}$).

Experiment 2: Effect of multiple injections on hypnosis time

Double and triple the $ED_{50}$ doses of JM-1232(-) (7.52 and 11.3 mg $kg^{-1}$), propofol (19.8 and 29.6 mg $kg^{-1}$) and the mixtures (0.5-mg $kg^{-1}$ JM-1232(-) and 3.5- and 5.3-mg $kg^{-1}$ propofol, 1.0-mg $kg^{-1}$ JM-1232(-) and 2.0- and 3.0-mg $kg^{-1}$ propofol) were administered. Each group was consisted of 6 animals. Immediately after recovery of the righting reflex, the same dose of the anesthetic that had been administered was repeated. Four injections were performed in each animal. The each hypnosis time after the injection was measured.

Experiment 3: Effect of flumazenil administered after the multiple injections on hypnosis time

After the same injections were performed as in experiment 2, the last (fourth) injection of the hypnotic drug was immediately followed by administration of 0.2-mg $kg^{-1}$ flumazenil (5 ml $kg^{-1}$). Each group was consisted of 6 animals. The each anesthesia time after the injection was measured and was compared the results of experiment 2.

The sample size of the study was determined following the previous investigation [12, 13].To analyze the 50% effective dose ($ED_{50}$) and the 95% confidence interval (CI) for loss of the righting reflex, we determined the number of animals that lost the righting reflex from the total that received an assigned pharmacological treatment and correlated the results with the probability of being under hypnosis using nonlinear least-squares logistic regression. The results for the required dose of propofol for each group are presented as the $ED_{50}$ and 95% CI.

Hypnosis time is expressed as the mean and SD. Analysis of variance (ANOVA) was used to compare the hypnosis time among groups, and the Newman-Keuls post hoc multiple-comparison test was used when ANOVA showed a statistically significant difference ($P < 0.05$). All calculations were performed using a statistical software package (SPSS 24, IBM Japan, Tokyo, Japan).

# Results

The rate of successful injection was totally 85%. Although a few animals showed a sign of temporal respiratory depression (hypopnea) immediately after the injection, there was no complication of animal death during the study.

Experiment 1: Interaction between JM-1232(-) and propofol

The percentage ratio of achieving hypnosis is shown in the Table. The hypnotic dose was 3.76 [3.36 - 4.10] mg $kg^{-1}$ ($ED_{50}$ and [95% confidence interval]) for JM-1232(-) and 9.88 [8.03 - 11.58] mg $kg^{-1}$ for propofol. Co-administration of 0.5, 1 and 2 mg $kg^{-1}$ JM-1232(-) reduced the hypnotic dose of propofol to 1.76 [1.21 - 2.51], 1.00 [0.46 - 1.86] and 0.44 [-0.38 - 0.80] mg $kg^{-1}$, respectively. The sum of the normalized doses of the mixtures [14] was 0.30, 0.35 and 0.54, and all values were under 0.9. Isobologram demonstrated that the $ED_{50}$ plots of the combinations were below the additivity line (Fig. 1).

Experiment 2: Effect of multiple injections on hypnosis time

The animals who received JM-1232(-) alone and the JM-1232(-)-propofol mixtures demonstrated significantly shorter recovery times after the first injection than those who received propofol alone at both double and triple $ED_{50}$ doses (Fig. 2). Hypnosis time correlated with the dose in all groups. With repeated injections, hypnosis time was prolonged in correlation with the repetition, except in the low dose propofol group (Fig. 3). The prolongation was more apparent in the groups administered JM-1232(-) alone.

Experiment 3: Effect of flumazenil administered after the multiple injections on hypnosis time

The hypnosis time of the first three injections in each group was consistent with the results of experiment 2. Administration of flumazenil immediately after the forth injection demonstrated no effect on the hypnosis time of the propofol alone groups, whereas the hypnosis times that had been extended by multiple injections of JM-1232(-) and the mixtures were significantly shortened by the administration of flumazenil (Fig. 4).

# Discussion

The results of the present investigation demonstrated that the new combination of JM-1232(-) and propofol shows significant supra-additivity of the hypnotic effect with a shorter recovery time than each drug administered alone. Despite the high potency of the mixtures, prolongation of the hypnosis time after multiple repeated injections seemed to be negligible. Although the pharmacokinetic properties, i.e., the drug concentration, were not determined, the results were clearly shown.

Drug interactions resulting from the pharmacokinetic and pharmacodynamic effects of drugs are one of the foci of anesthesiology [14, 15]. General anesthesia consists of two factors, hypnosis and analgesia. It is well known that the combination of multiple agents, such as anesthetics and opioids, synergistically enhances the potency of general anesthesia [16 - 18]. Although most studies have focused on the interactions between hypnotics and analgesics, the interaction within an area of hypnosis itself leaves

room for investigation searching new practices. On the other hand, multimodal analgesia using a combination of drugs has become popular in the field anesthesiology [19].

Some barbiturate derivatives are associated with rapid recovery from hypnosis, but, their repeated and prolonged administration delays emergence from anesthesia [20]. The combining drugs might provide not only supra-additivity, but also quick recovery profiles independent of contextual uses of anesthetics. JM-1232(-) demonstrated a shorter recovery time than propofol at the first administration; however, with multiple injections, recovery time was prolonged and exceeded that of propofol. On the other hand, the mixture of JM-1232(-) and propofol resulted in minimal prolongation of recovery time even after multiple injections in the current investigation.

Even after repeated injections of the combination, flumazenil completely abolished the synergistic interaction, resulting in recovery of the pharmacodynamic profile. Although it is possible that flumazenil might partially activate the benzodiazepine receptor [21, 22] and potentiate the hypnotic potency of anesthetics [12, 23], our results showed a sufficient reversal effect of flumazenil.

The limitations of the current study should be addressed. We could not predict the precise properties of the interaction, thus, the dose of drugs and sample size of the study was determined following the results of our previous investigation with extrapolation [13, 23].

Diazepam is a more popular benzodiazepine, but a lipophilic agent and insoluble in water, while midazolam is a water soluble benzodiazepine. However, our preliminary experiments showed that the potency of midazolam in mice is very low, which is similar to the results of Kilpatrick et al. [24]. Thus, the highly effective JM-1232(-) was chosen as the supplemental drug for co-administration with propofol in the current investigation. Co-administration of midazolam could decrease the time to achieve hypnosis without delaying emergence during short-term propofol anesthesia in daily clinical settings [10, 23]. Not only JM-1232(-), but other benzodiazepines as well might produce similar results as in the present study. However, JM-1232(-) has a short duration of action, which makes it more suitable for use as a supplementary drug [25].

Another limitation was that we evaluated only the dose required for achieving hypnosis and the hypnosis time. Due to technical problems, the blood concentration of each drug was not determined. Electroencephalographic analysis might be useful for comparing the synergistic effects of drugs from the pharmacodynamic point of view [26, 27]. Further investigation is, therefore, required.

# Conclusions

In summary, the new combination, JM-1232(-) and propofol, demonstrated ultra-short acting hypnotic effects. The supra-additive interaction could lead to the development of a new general anesthetic regimen.

# Abbreviations

ddy: Deutsch-Denken-Yoken, $ED_{50}$: 50% effective dose, CI: confidence interval, ANOVA: Analysis of variance

# Declarations

Declarations

Ethics approval and consent to participate

The study was approved by Animal Experiment Committee of Nagoya University Graduate School of Medicine, 26401.

Consent to participate is not applicable for the study.

Consent for publication

Not applicable for the study.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

ST performed the experiment, writing the manuscript.

MH helped to writing and correction of the manuscript and conducting the study.

NM helped and directed study.

MS organized the study and writing the manuscript.

TT performed the experiment and writing the manuscript.

YUA conducted the study design, performed the experiments and data analysis, and writing the current manuscript.

ASB directed the study and revised the manuscript.

MO organized the study and examined pharmacological procedures.

All authors have read and approved the manuscript.

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# Tables

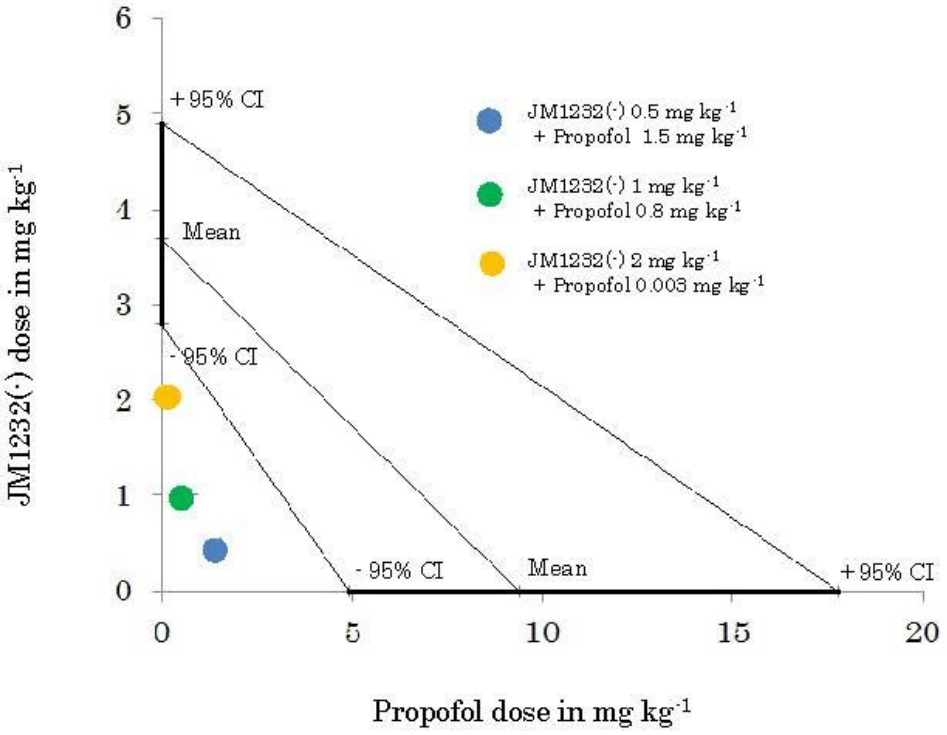
Table. The percent ratios of responders in each treatment.

| | Dose of JM-1232 (mg $kg^{-1}$) | | | | |
|---|---|---|---|---|---|
| | 3 | 3.5 | 4 | 4.5 | 5 |
| JM-1232(-) alone | 0 | 33 | 83 | 83 | 100 |

| | Dose of propofol (mg $kg^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0.625 | 1.25 | 2.5 | 3.75 | 5 | 7.5 | 10 | 12.5 | 15 |
| Propofol alone | | | | | 0 | 17 | 67 | 67 | 100 |
| Combination of Propofol and JM-1232(-) | | | | | | | | | |
| JM-1232(-) 0.05 mg $kg^{-1}$ | 0 | 33 | 83 | 100 | | | | | |
| JM-1232(-) 0.1 mg $kg^{-1}$ | 50 | 50 | 100 | | | | | | |
| JM-1232(-) 0.2 mg $kg^{-1}$ | 83 | 100 | 100 | | | | | | |

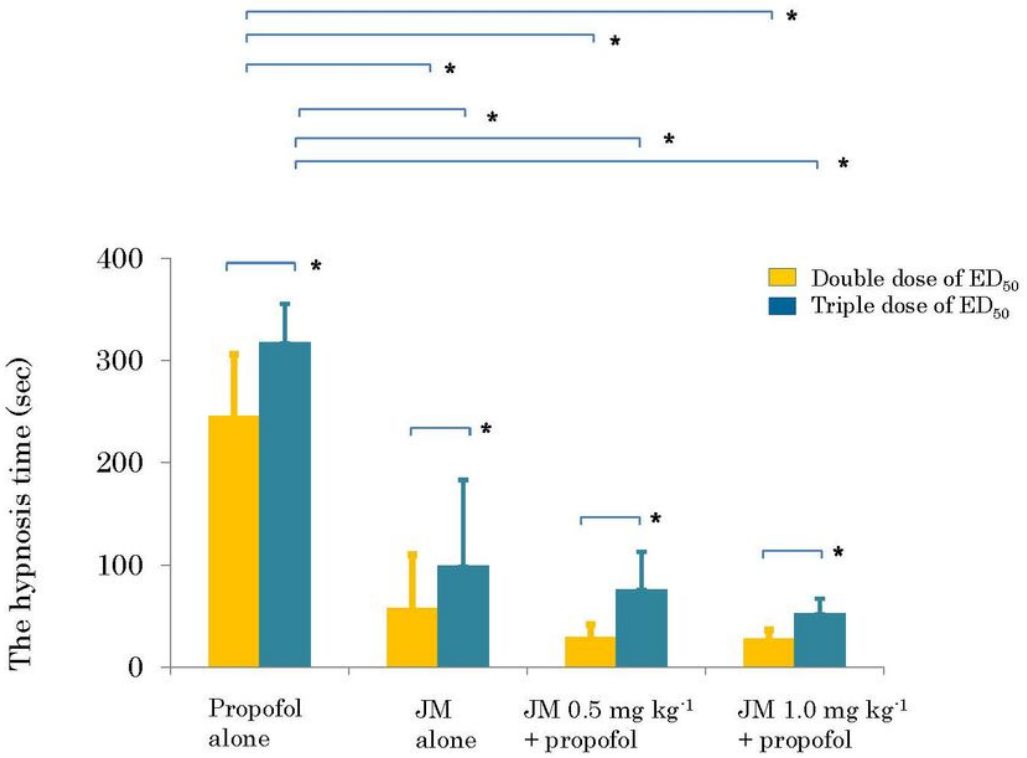
The ratios of responders to total number of animals (n = 6) are expressed as the percentage (%).

# Figures



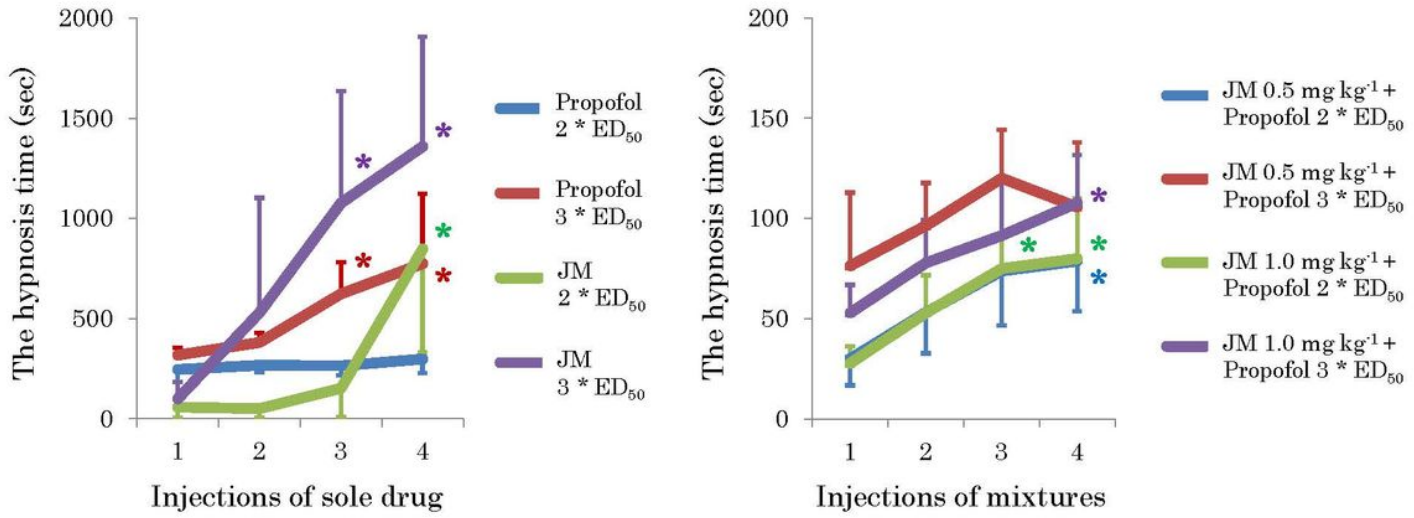

## Figure 1

(a.) Isobologram for the JM1232(-) and propofol combination. CI: confidence interval. (b.) Duration of loss of the righting reflex (mean ± SD). Groups in which all animals (n=6) lost the righting reflex with the lowest dose of the drugs administered were analyzed. There were no significant differences between the groups.




## Figure 2

Righting reflex recovery time after the first injection (anesthesia time). The data are demonstrated as mean and SD. All larger dose administrations prolonged anesthesia time. JM-1232(-) (JM) demonstrated a shorter recovery time and the mixtures demonstrated more prompt emergence from anesthesia. ED50: 50% effective dose; JM: JM1232(-); *: $P < 0.05$ between groups.




## Figure 3

Change in anesthesia time with repeated injections of 2- and 3-fold the ED50 doses of each of the study drugs, JM-1232(-) (JM), propofol and mixtures of JM-1232(-) and propofol. Repeat injections significantly extended recovery time after the administration of JM-1232(-) and larger doses of propofol. *: $P < 0.05$ vs. each anesthesia time after the first injection.

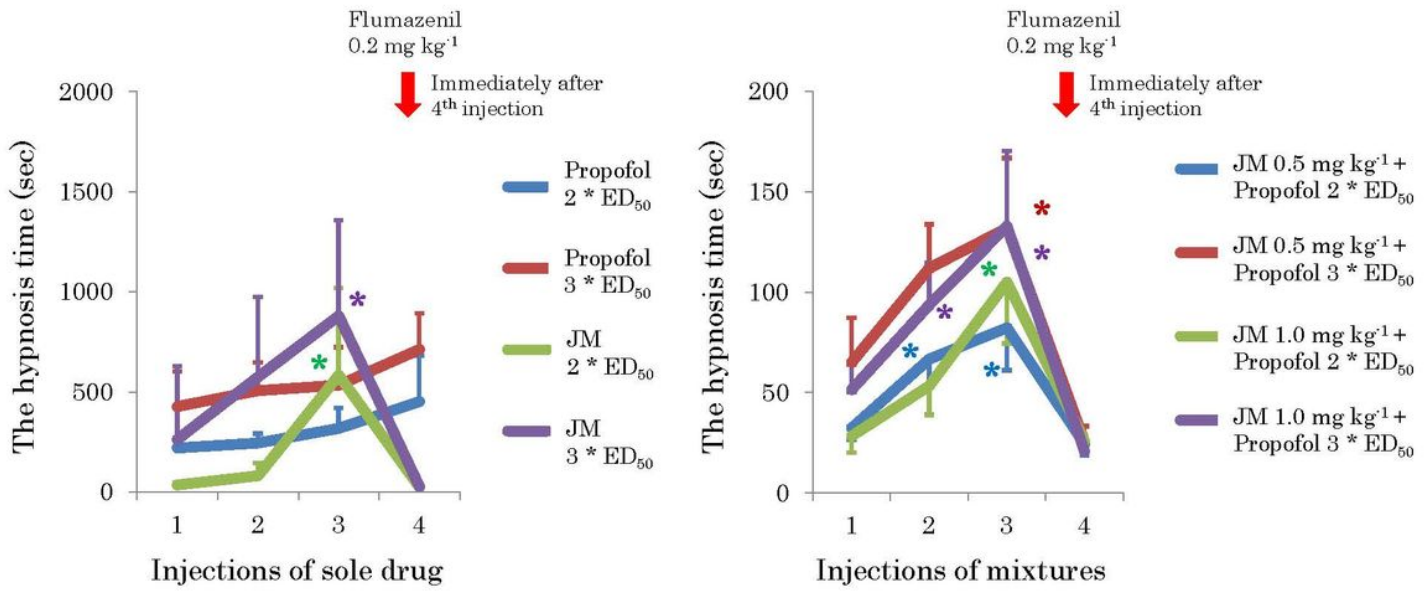



**Figure 4**

Change in anesthesia time with repeated injections of 2- and 3-fold the ED50 doses of each of the study drugs, N-1232(-) (JM), propofol and mixtures of JM-1232(-) and propofol, and recovery with flumazenil. Repeat injections significantly extended recovery time after the administration of JM-1232(-) and larger doses of propofol. Supplementary administration of flumazenil significantly shortened the recovery time except in the groups given propofol alone. *: $P < 0.05$ vs. each anesthesia time after the first injection.

# Supplementary Files

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