



Original

JM-1232(-) and propofol, a new combination of hypnotics with short-acting and non-cumulative preferable properties

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Abstract: Drug interactions are significant in anesthesiology because drug combinations can potentially possess novel properties. The pharmacological advantages of a new combination of the benzodiazepine receptor agonist JM-1232(-) and propofol were investigated in mice. Male adult mice were administered JM-1232(-) or propofol or 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, doses double and triple the ED₅₀ of propofol were injected with JM-1232(-) to compare hypnosis time. The injections were repeated four times, and the hypnosis times were compared. Flumazenil was administered separately immediately after the last dose was injected. The ED₅₀ values ([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 0.5 and 1 mg kg⁻¹ JM-1232(-) reduced the ED₅₀ values of propofol to 1.76 [1.21–2.51] and 1.00 [0.46–1.86] mg kg⁻¹, respectively. The drug combination for hypnosis produced a supra-additive interaction. Hypnosis time was significantly shorter in the groups given the mixtures compared to each hypnotic administered alone. After repeated injections, hypnosis time with the mixtures showed smaller prolongation than that with the hypnotic alone. Flumazenil completely restored the recovery time after anesthesia. The combination of JM-1232(-) and propofol showed a supra-additive interaction, and the reduced hypnotic dose contributed to a faster recovery even after multiple injections.

Key words: hypnotics, flumazenil, JM-1232(-), propofol, supra-additive interaction

Introduction

JM-1232(-) is an isoindoline derivative developed in Japan and potential anesthetic with a short duration of

action [1, 2]. JM-1232(-) is water soluble and is highly potent. Although the molecular structure of JM-1232(-) is different from classical and typical benzodiazepines, JM-1232(-) enhances synaptic inhibition by modulating

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benzodiazepine binding sites on γ -aminobutyric acid A receptors, similar to benzodiazepine derivatives [3]. The characteristics of JM-1232(-) might be suitable for supporting not only laboratory experiments but also clinical anesthesia and intensive care [4]. JM-1232(-) has been administered to humans as “MR04A3,” and demonstrated favorable and acceptable profiles in a preclinical trial [5].

Propofol is one of the most popular intravenous anesthetics in daily clinical practice [6] including veterinary medicine. The characteristics of propofol, including rapid onset and prompt recovery, make it an appropriate drug for general anesthesia. However, a long-lasting infusion of propofol might lead to prolongation of its effect and delay recovery [7]. The dose of propofol required to induce anesthesia can be reduced by a series of pre-medications [8, 9]. Some of these drugs, such as benzodiazepine derivatives, significantly enhance the hypnotic activity of propofol [10, 11]. Thus, co-administration of JM-1232(-) could reduce the required dose of propofol. Moreover, the reduced propofol dose might lead to further quick recovery. The new properties including more potent, faster recovery, less accumulative, may be preferable for achieving hypnosis during not only induction of anesthesia but also maintenance of sedation. The combination of other analgesics, including opioids, would provide more appropriate anesthetic conditions.

In the current study, the anesthetic properties of JM-1232(-) and propofol was evaluated in an *in vivo* investigation using mice. The induction and recovery profiles were investigated after repeated injections of the drug mixture, which simulated prolonged infusion. Finally, the animals were administered flumazenil to assess its antagonistic effects for recovery after long-lasting hypnosis.

Materials and Methods

Animals

After obtaining approval from the Ethics Committee for Animal Experiments at our institution (Final registration number: 26401), all experiments were performed in an animal laboratory. Male adult Deutsch-Denken-Yoken (ddY, closed colony) mice weighing 38 to 45 g (SLC Japan, Hamamatsu, Japan) were used. The animals were maintained on 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.

Drugs

Propofol (Diprivan, AstraZeneca K.K., Osaka, Japan) was diluted with 10% soybean oil (Intralipid, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). JM-1232(-), which was provided as a gift from Maruishi Pharmaceutical (Osaka, Japan), was 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 were calculated based on past and preliminary experiments [12, 13] (Table 1). The ED₅₀ of propofol was first tested, and then other combinations were tried until all animals in the group showed the same response (hypnosis or not).

Procedures

The mice were set in a transparent animal holder to place a 24 G 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-sy-

Table 1. The percentage ratios of achieving hypnosis 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(-) 0.5 mg kg ⁻¹	0	33	83	100						
JM-1232(-) 1.0 mg kg ⁻¹	50	50	100							
JM-1232(-) 2.0 mg kg ⁻¹	83	100	100							

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

ringe was set into the plastic cannula, and the prepared material was quickly injected over 2–3 s. If the injection was irregular and incomplete, such as being resistant or showing extravasation, the mouse was omitted from the study. Mice were released from the animal holder and individually evaluated for hypnosis on a flat heated (35°C) 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 the end of hypnosis was defined as hypnosis time. Hypnosis time was recorded in the laboratory room and was verified by another blinded technical assistant using recorded movies of the experiments on another day. After the experiments, all animals were killed with inhalation of pure carbon dioxide more than 5 min following to the regulation of our institute.

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 consisted of six 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 ED₅₀ 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. The doses were set as sufficient administration for achieving hypnosis in all animals. Each group consisted of six animals. Immediately after recovery of the righting reflex, the same dose of the anesthetic that had been administered was repeated. Four injections were given to each animal. Hypnosis time was measured after the injection.

Experiment 3: Effect of flumazenil administered after 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 consisted of six animals. Anesthesia time after the injection was measured and was compared with the results of experiment 2.

Statistical analysis

The sample size of the study was determined following a previous investigation [12, 13]. To analyze the 50% effective dose (ED₅₀) and the 95% confidence intervals (CIs) 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 their being under hypnosis using nonlinear least-squares logistic regression. The results for the required propofol dose for each group are presented as the ED₅₀ and 95% CI.

Hypnosis time is expressed as mean ± 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 significant difference (*P*<0.05). The validity of the multiple-comparison test was verified in the previous investigation in comparison with other tests including Tukey-Kramer method [12, 13]. All calculations were performed using a statistical software package (SPSS 24, IBM Japan, Tokyo, Japan).

Results

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

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

The percentage ratio of achieving hypnosis is shown in Table. The hypnotic dose was 3.76 [3.36–4.10] mg kg⁻¹ (ED₅₀ and [95% CI]) 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 sums of the normalized doses of the mixtures [14] were 0.30, 0.35, and 0.54, and all values were under 0.9. The isobologram demonstrated that the ED₅₀ plots of the combinations were below the additivity line (Fig. 1).

Experiment 2: Effect of multiple injections on hypnosis time

Animals who received JM-1232(-) alone and the JM-

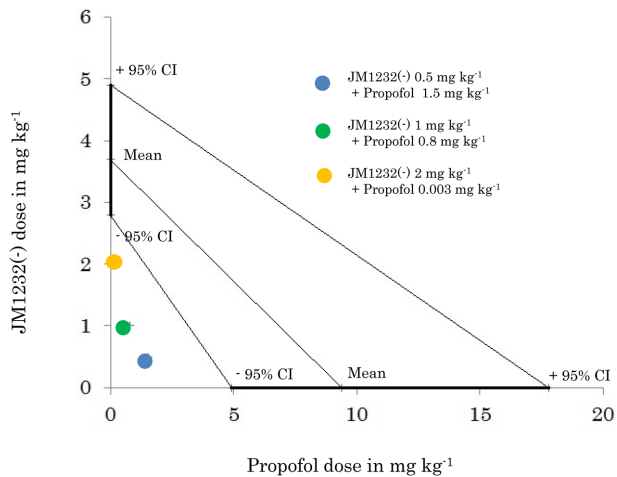


Fig. 1. Isobologram for the JM1232 (-) and propofol combination. CI: confidence interval.

1232(-)-propofol mixtures demonstrated significantly shorter recovery times after the first injection than those that received propofol alone at both double and triple the ED_{50} dose (Fig. 2). Hypnosis time was correlated with the dose in all groups. The prolongation of hypnosis time was correlated with repeated injections, 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 fourth injection demonstrated no effect on the hypnosis time in 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 administration of flumazenil (Fig. 4).

Discussion

The present investigation demonstrated that the new combination of JM-1232(-) and propofol showed significant supra-additivity of the hypnotic effect with a shorter recovery time than either 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 demonstrated.

Drug interactions resulting from the pharmacokinetic and pharmacodynamic effects of drugs are one of the foci of anesthesiology [14, 15]. General anesthesia con-

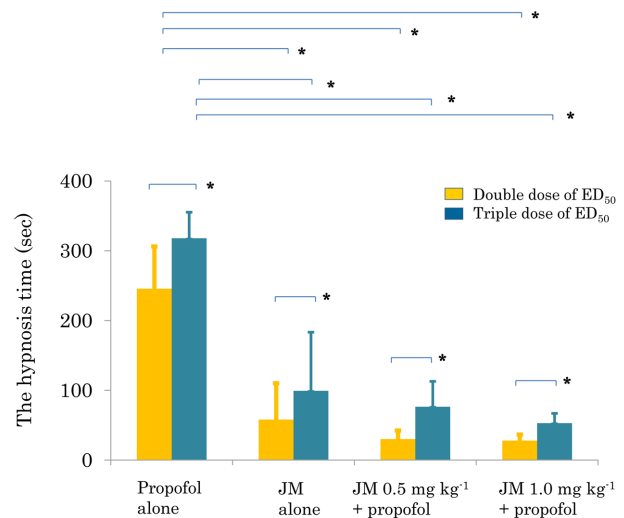


Fig. 2. Righting reflex recovery time after the first injection (anesthesia time). The data are demonstrated as mean and SD. All larger dose administrations prolonged anesthesia time. JM-1232(-) (JM) demonstrated a shorter recovery time and the mixtures demonstrated more prompt emergence from anesthesia. ED_{50} : 50% effective dose; JM: JM1232 (-); *: $P < 0.05$ between groups.

sists of 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 interactions between hypnotics and analgesics, interactions within in the domain of hypnotics leaves room to search for new practices. On the other hand, multimodal analgesia using a combination of drugs has become popular in the field of anesthesiology [19]. When the pharmacokinetics of each drug was not affected, the synergistic effect could reduce the each absolute dose of drug, thus, the reduced administration might shorten residual and extending effect not only after the first infusion but also after the multiple infusions.

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

Furthermore, flumazenil completely abolished the synergistic interaction, even after repeated injections of the combination, resulting in recovery of the pharmacodynamic profile. Although it is possible that flumazenil

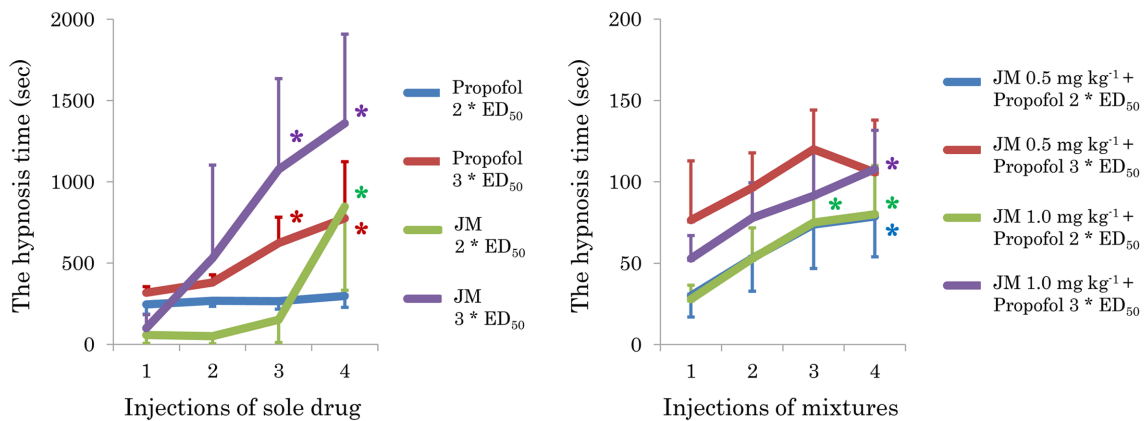


Fig. 3. Change in anesthesia time with repeated injections of 2- and 3-fold the ED_{50} doses of each of the study drugs, The data are demonstrated as mean and SD. JM-1232(-) (JM), propofol and mixtures of JM-1232(-) and propofol. Repeat injections significantly extended recovery time after the administration of JM-1232(-) and larger doses of propofol. *: $P < 0.05$ vs. each anesthesia time after the first injection.

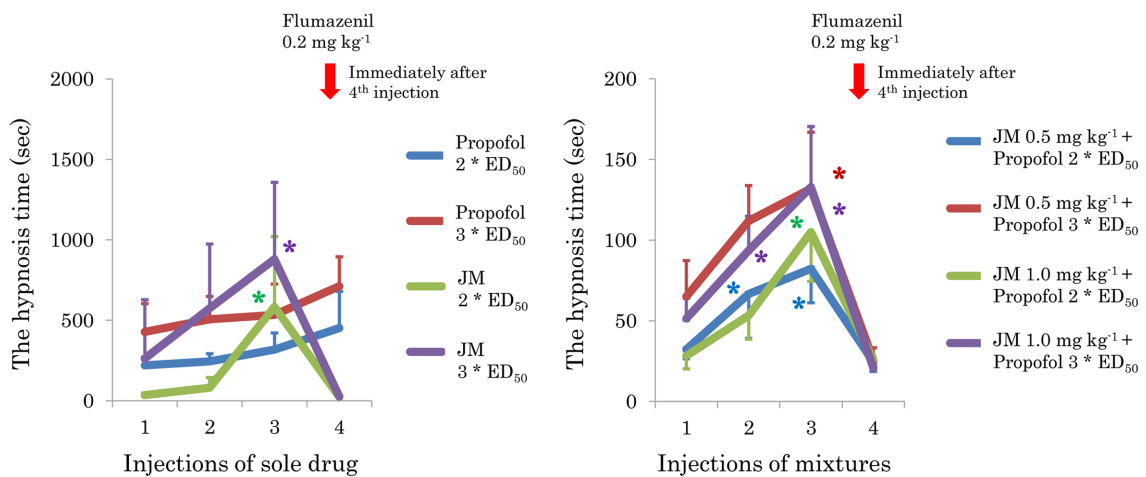


Fig. 4. Change in anesthesia time with repeated injections of 2- and 3-fold the ED_{50} doses of each of the study drugs, N-1232 (-) (JM), propofol and mixtures of JM-1232(-) and propofol, and recovery with flumazenil. The data are demonstrated as mean and SD. Repeat injections significantly extended 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.

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.

Diazepam is a popular benzodiazepine, but a lipophilic agent and insoluble in water, whereas midazolam is a water soluble benzodiazepine. However, our pilot experiments showed that the potency of midazolam in mice is very low, which is consistent 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 decreases the time to achieve hypnosis without delaying emergence during short-term propofol anesthesia in daily clinical settings [11, 23]. Not only JM-1232(-) but also other benzodi-

azepines might well produce similar results to those seen 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].

The limitations of the current study should be addressed. We could not predict the precise properties of the interaction; thus, the dose of the drugs and the sample size of the study were determined following the results of our previous investigation with extrapolation [13, 23]. Another limitation was that we only evaluated the dose required to achieve hypnosis and the hypnosis time. Due to technical problems, the blood concentration of each drug was not determined. An electroencephalographic analysis might be useful to compare the synergistic effects of the drugs from a pharmacodynamic point of view [26, 27]. Finally, the volume of injection might be large for

the animals. The results of the current drug interaction, however, might not be affected by the volume.

Conclusions

In summary, a new combination of 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 with analgesics, including opioids. The new properties of the combination might be appropriate for every interventions requiring hypnosis.

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.

Conflicts of Interests

The authors declare that they have no competing interests

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Authors' Contributions

ST performed the experiment, writing the manuscript. TT performed the experiment and 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. YUA conducted the study design, performed the experiments and data analysis, and writing the cur-

rent 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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