J Anesth (2015) 29:798–802
DOI 10.1007/s00540-015-2017-9

SHORT COMMUNICATION

Effects of topical and intravenous JM-1232(−) infusion on cerebrovascular reactivity in rats

Kenji Iwata[1] · Hiroki Iida[1] · Mami Iida[2] · Naokazu Fukuoka[1] · Kazuhiro Kito[1] · Kumiko Tanabe[1]

Received: 9 September 2014 / Accepted: 15 April 2015 / Published online: 26 April 2015

Abstract A novel short-acting benzodiazepine receptor agonist, JM-1232(−), has been shown to have a sedative/hypnotic effect and wide safety margin. However, its effect on cerebral vessels is not well known. Therefore, we investigated the cerebrovascular reactivity to topical and intravenous JM-1232(−) and during hypotension or hypercapnia with intravenous administration of JM-1232(−). We used a closed cranial window preparation to measure the changes of cerebral pial arteriolar diameters in isoflurane-anesthetized Sprague–Dawley rats. We first measured the direct effect of topical JM-1232(−). We then determined the effect of intravenous JM-1232(−) and then we measured the response to hypercapnia before and after JM-1232(−) infusion. Finally, we measured the reaction to stepwise induction of hypotension before and after JM-1232(−) infusion. Topical infusion of JM-1232(−) dilated pial arterioles. Intravenous infusion of JM-1232(−) changed pial arterioles by 4.5 ± 2.7 %, 5.0 ± 3.9 %, and −2.8 ± 2.6 % (at 0.1, 0.3, and 1.0 mg/kg/min, respectively). Hypercapnia dilated pial arterioles before and after JM-1232(−) infusion. The diameters of pial arterioles did not change during hypotension before or after intravenous JM-1232(−) infusion. These results indicate that topical JM-1232(−) has a dilative effect on pial arterioles and that intravenous administration of JM-1232(−) may not affect cerebrovascular reactivity to hypotension or hypercapnia.

✉ Hiroki Iida
iida@gifu-u.ac.jp

[1] Department of Anesthesiology and Pain Medicine, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu-City, Gifu 501-1194, Japan

[2] Division of Internal Medicine and Cardiology, Gifu Prefectural General Medical Center, Gifu, Japan

Keywords JM-1232(−) · Cerebrovascular reactivity · Cranial window

JM-1232(−) is a novel isoindoline water-soluble benzodiazepine receptor agonist, which induces a sedative/hypnotic effect [1]. It has recently been used for human treatment [2], but only a few reports regarding its effect on human vessels are available. For example, JM-1232(−) has been reported to have dilative potential for the human gastroepiploic artery [3], but its effect on cerebral pial arterioles has not yet been investigated. It is also uncertain as to whether JM-1232(−) is safe for the sedation of severely ill patients with neurosurgical disorders because its effect on the cerebral vascular response to hypercapnia or systemic hypotension is not known. Thus, we used a closed cranial window method to investigate the JM-1232(−) modulation of pial arteriolar responses to physiological changes in rats.

We studied 18 male Sprague–Dawley rats weighing 300–400 g. The experimental protocols were approved by the Institutional Committee for Animal Care of Gifu University Graduate School of Medicine. All animals were anesthetized with intraperitoneal sodium pentobarbital (50 mg/kg). They were mechanically ventilated through a tracheotomy using a ventilator (Model 683, Harvard Apparatus, Holliston, MA) with room air supplemented with oxygen. The respiratory rate was set at 60/min, and tidal volume was adjusted to maintain the $PaCO_2$ between 35 and 40 mmHg. The inspired oxygen fraction was maintained at approximately 0.4. Anesthesia was maintained with isoflurane (end-tidal concentration 1–1.2 %). A femoral artery was cannulated to measure the mean arterial blood pressure (MABP), withdraw or re-infuse blood (to induce systemic hypotension), and analyze arterial blood gas tensions and pH. A femoral vein was cannulated for

experimental drug administration. Rectal temperature was maintained between 37 and 38 °C using a heating pad. A closed cranial window preparation was used for observation of the pial vessels, as described previously [4]. The window was filled with artificial cerebrospinal fluid (aCSF; as previously described) [4]. The window had three catheters: the first catheter was attached to a reservoir bottle containing aCSF to maintain constant intracranial pressure and wash out the experimental drugs with aCSF, the second was used to drain the fluid from the window, and the third was used to administer experimental drugs. The temperature within the window was monitored using a thermometer (Model 6510; Mallincrodt Medical; St. Louis, MO, USA) and maintained between 37 and 38 °C. The pial views obtained in these experiments were stored on videotape (with a time record). In each rat, the diameters of three pial arterioles (baseline diameters, 32–110 μm) were measured using a videomicrometer (Model VM-50 N, Olympus, Tokyo, Japan) on a television monitor that received signals from a microscope (M165C, Leica Microsystems, Wetzlar, Germany). The vessels that were to be used to collect data were always selected at the beginning of the experimental period. In each rat, the responses of the three arterioles were averaged, and these averaged responses were used to obtain group means. In experiment 1, the direct effect of JM-1232(−) on pial arterioles (topical administration) was studied ($n = 6$ rats). Each concentration of JM-1232(−) (10^{-8} M, 10^{-6} M, and 10^{-4} M) was administered through the window, and pial arteriolar diameters were measured before and after the test drug administration. Topical JM-1232(−) was administered at 0.2 ml/min for 5 min. Thus, the volume of drug of each concentration was 1 ml, respectively. We measured the pial arteriolar diameter around 4 min after the start of infusion, similar to a previous study [5]. Each drug dose was administered after a 20-min wash-out period with aCSF. In experiment 2, the dose–response to JM-1232(−) and its influence on the hypercapnic response were investigated ($n = 6$ rats). Under control conditions, without JM-1232(−), hypercapnia was induced by lowering the respiratory rate until the end-tidal CO_2 partial pressure increased to approximately 60 mmHg. Hypercapnia was maintained for 5 min and then pial arteriolar diameters were measured. The diameters (after hypercapnia) were compared to the baseline diameter (before hypercapnia) of the same arterioles. After confirming the baseline response to hypercapnia, JM-1232(−) was infused intravenously at three incremental rates (first, 0.1 mg/kg/min; next, 0.3 mg/kg/min; and finally, 1.0 mg/kg/min; each for 20 min). The dose–response of the pial arterioles to intravenous JM-1232(−) was examined by comparing the post-treatment diameters to those before intravenous administration. The influence of JM-1232(−) on the hypercapnic response was examined by measuring pial arteriolar diameters 20 min after beginning the infusion of JM-1232(−) at 1.0 mg/kg/min (before hypercapnia) and 5 min after hypercapnia induction, which was performed as described above. In experiment 3 ($n = 6$ rats), stepwise hypotension was achieved by withdrawing arterial blood every 2 min (1 ml each) until the blood pressure decreased to 50 % of the control. The blood was subsequently reinfused every 2 min (1 ml each). Pial arteriolar diameters were measured before and after the stepwise hypotension, followed by intravenous infusion of 1.0 mg/kg/min JM-1232(−). Twenty minutes after beginning the infusion, stepwise hypotension and pial arteriolar diameter measurements were performed in the above-mentioned manner. Changes in all variables were examined by a one-way analysis of variance (ANOVA) for repeated measurements with a Bonferroni correction for post hoc comparison. The dose-dependent effect of JM-1232(−) was examined by a one-way ANOVA with a Bonferroni correction for post hoc comparison. The effect of presence of JM-1232(−) was examined by the paired t test. Significance was set at $p < 0.05$. All values are presented as mean ± SD.

In experiment 1, topical JM-1232(−) administered at 10^{-8}, 10^{-6}, and 10^{-4} M dilated pial arteriolar diameter by 3.6 ± 1.6, 8.7 ± 1.9, and 9.9 ± 4.3 %, respectively (Fig. 1a). In experiment 2, intravenous infusion of 1.0 mg/kg/min JM-1232(−) strongly lowered the MABP, but did not increase the heart rate. Intravenous infusion of 0.1 and 0.3 mg/kg/min JM-1232(−) dilated the pial arteriolar diameter by 4.5 ± 2.7 % ($p = 0.01$) and 5.0 ± 3.9 % ($p = 0.03$), respectively, compared to the pre-infusion state. Intravenous infusion of 1.0 mg/kg/min of JM-1232(−) changed pial arteriolar diameter by −2.8 ± 2.6 % ($p = 0.35$) compared to the pre-infusion state (Fig. 1b). Under the control condition, hypercapnia dilated pial arteriolar diameter by 8.1 ± 1.3 % compared to normocapnia ($p < 0.001$). Under the JM-1232(−)-infused condition, although the MABP decreased from 116 to 72 mmHg, hypercapnia dilated pial arteriolar diameter by only 8.1 ± 1.9 % compared to normocapnia, which was not significantly different from the control condition response (Fig. 1c). In experiment 3, under control conditions, systemic hypotension, which was induced by withdrawing blood, changed the pial arteriolar diameter by 0.4 ± 3.5 %. Intravenous infusion of 1.0 mg/kg/min JM-1232(−) decreased the MABP from 110 to 59 mmHg. Pial arteriolar diameter was slightly changed (by −2.4 ± 7.2 %) after JM-1232(−) infusion (pre-blood withdrawal). After intravenous infusion of JM-1232(−), further induction of systemic hypotension by blood withdrawal (the MABP decreased further from 59 to 33 mmHg) dilated pial arteriolar diameter by 1.7 ± 10.8 %. This response did not significantly differ from that observed under the control conditions (Fig. 1d).

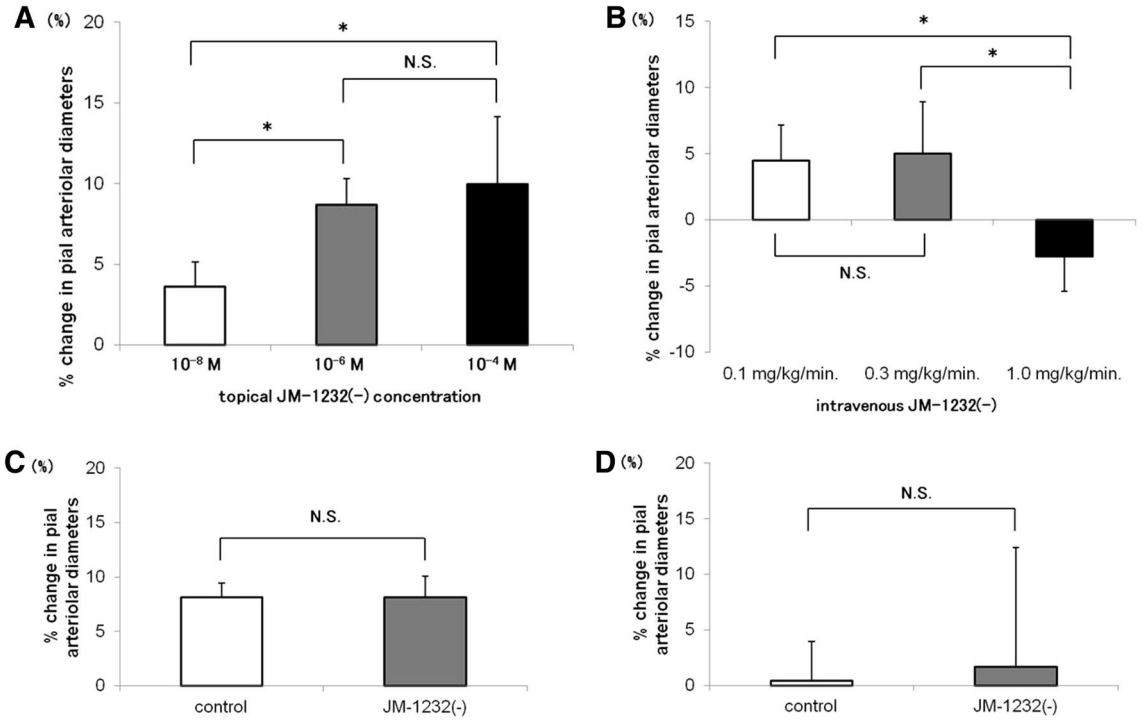

Fig. 1 Topical infusion of JM-1232(−) (10^{-8}, 10^{-6}, and 10^{-4} M) induced vasodilation of pial arterioles (3.6 ± 1.6, 8.7 ± 1.9, and 9.9 ± 4.3 %, respectively) (**a**). Intravenous infusion of JM-1232(−) dilated pial arteriolar diameter by 4.5 ± 2.7 and 5.0 ± 3.9 % at 0.1 and 0.3 mg/kg/min, respectively, but the dilative response was not observed at 1.0 mg/kg/min (**b**). Under the control conditions, hypercapnia dilated pial arteriolar diameter by 8.1 ± 1.3 %. Under JM-1232(−)-infused conditions, a hypercapnic response of 8.1 ± 1.9 % was observed (**c**). Under the control conditions, systemic hypotension induced pial arteriolar diameter change by 0.4 ± 3.5 %. Under JM-1232(−)-infused conditions, systemic hypotension induced a pial arteriolar change by 1.7 ± 10.8 % (**d**). *N.S.* not significant, * $p < 0.05$

Hemodynamic and physiological measurements for each experiment are shown in Table 1. Intra-window and rectal temperature were kept under slightly mild hypothermia.

The following were the major findings in the present study: (1) topical JM-1232(−) has a dilative effect on pial arterioles, (2) intravenous infusion of 0.1 and 0.3 mg/kg/min JM-1232(−) has a slight dilative effect on pial arterioles, and (3) intravenous infusion of 1.0 mg/kg/min JM-1232(−) does not affect pial arteriolar response during hypercapnia or systemic hypotension.

In a previous study that used a human gastroepiploic artery graft, JM-1232(−) demonstrated a dose-dependent dilative potential [3]. In the present study, intravenous infusion of 0.1 and 0.3 mg/kg/min and topical infusion of JM-1232(−) in rats had dilative effects on pial arterioles (by 4.5–9.9 %) in vivo under isoflurane anesthesia. However, infusion of 1.0 mg/kg/min JM-1232(−) did not dilate pial arterioles. To our knowledge, the effect of JM-1232(−) on cerebral metabolic flow coupling has not yet been defined. Although the precise mechanism of lack of vasodilation is unclear, it may be due to a reduced cerebral metabolic rate and/or systemic hypotension induced by higher doses of JM-1232(−). Other sedative agents such as midazolam, propofol, and thiopental have different effects on pial arterioles. Although intravenous administration of midazolam does not affect pial arteriolar diameter in cats [6], topical propofol at 10^{-4} M has a dilative effect on pial arterioles by 5.9 % in rabbits [7]. In addition, thiopental has no dilative or constrictive effect on rat aortic rings [8]. On the other hand, volatile anesthetics, such as 1 minimum alveolar concentration isoflurane and sevoflurane, have dilative effects on pial arterioles (by 19 and 14 %, respectively) in dogs [9]. In the present study, JM-1232(−) was used with basal isoflurane anesthesia. Thus, the results were not compared directly to other anesthetics. However, it is clear that JM-1232(−) has some vasodilative potential in the range of a certain concentration.

Although intravenous infusion of 1.0 mg/kg/min JM-1232(−) decreased MABP to 50 % of the control, JM-1232(−) added to isoflurane did not affect pial arteriolar diameters. Furthermore, under background anesthesia of isoflurane, JM-1232(−) did not affect the pial arteriolar dilative response to hypercapnia. Both midazolam [10, 11] and propofol [11, 12] were shown to maintain cerebral CO_2 response. With regard to its response to hypercapnia,

Table 1 Hemodynamic and physiological measurements during experiments

	HR (bpm)	MABP (mmHg)	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	RT (°C)	WT (°C)
Experiment 1 ($n = 6$)							
Pre JM-1232(−) 10^{-8} M	372 ± 18	112 ± 9	7.42 ± 0.01	36.1 ± 1.2	219 ± 46	36.5 ± 0.7	36.4 ± 1.1
Post JM-1232(−) 10^{-8} M	375 ± 18	111 ± 9					
Pre JM-1232(−) 10^{-6} M	384 ± 11	112 ± 8	7.41 ± 0.01	37.3 ± 1.4	213 ± 48	37.0 ± 0.7	36.3 ± 0.8
Post JM-1232(−) 10^{-6} M	386 ± 12	113 ± 8					
Pre JM-1232(−) 10^{-4} M	395 ± 19	113 ± 10	7.41 ± 0.01	37.8 ± 1.7	205 ± 51	37.0 ± 0.7	36.6 ± 0.8
Post JM-1232(−) 10^{-4} M	396 ± 23	113 ± 11					
Experiment 2 ($n = 6$)							
Pre JM-1232(−) infusion	384 ± 58	123 ± 10	7.41 ± 0.02	37.2 ± 2.7	187 ± 44	37.2 ± 0.6	36.4 ± 0.3
0.1 mg/kg/min. JM-1232(−)	376 ± 56	116 ± 14	7.41 ± 0.03	36.2 ± 1.5	182 ± 44	37.3 ± 0.6	36.7 ± 0.4
0.3 mg/kg/min. JM-1232(−)	404 ± 70	103 ± 11	7.41 ± 0.03	35.5 ± 1.6	179 ± 45	37.2 ± 0.6	36.2 ± 0.4
1.0 mg/kg/min. JM-1232(−)	393 ± 70	72 ± 17‡	7.39 ± 0.03	36.1 ± 2.2	177 ± 44	37.0 ± 0.4	36.7 ± 0.2
Pre hypercapnia, control	387 ± 80	116 ± 11	7.40 ± 0.03	37.3 ± 0.9	199 ± 37	36.9 ± 0.7	36.1 ± 0.6
Post hypercapnia, control	377 ± 70	119 ± 11	7.23 ± 0.01*	63.4 ± 4.3*	156 ± 48*	37.2 ± 0.5	36.4 ± 0.7
Pre hypercapnia, JM-1232(−)	393 ± 70	72 ± 17*†	7.39 ± 0.03†	36.1 ± 2.2†	177 ± 44	37.0 ± 0.4	36.7 ± 0.2
Post hypercapnia, JM-1232(−)	393 ± 78	68 ± 14*†	7.20 ± 0.02*#	63.3 ± 4.7*#	146 ± 52*#	37.2 ± 0.5	36.9 ± 0.5
Experiment 3 ($n = 6$)							
Pre hypotension, control	375 ± 24	110 ± 5	7.41 ± 0.03	37.0 ± 1.5	200 ± 43	37.2 ± 0.6	36.7 ± 0.6
Post hypotension, control	325 ± 35	47 ± 6*	7.42 ± 0.01	34.3 ± 2.8	211 ± 35		
Pre hypotension, JM-1232(−)	344 ± 50	59 ± 7*†	7.39 ± 0.02	36.7 ± 3.1	196 ± 37	36.6 ± 0.8	36.6 ± 0.4
Post hypotension, JM-1232(−)	290 ± 22*#	33 ± 1‡	7.41 ± 0.02	33.2 ± 4.8	193 ± 45		

Values are presented as mean ± SD

HR heart rate, *MABP* mean arterial blood pressure, *RT* rectal temperature, *WT* intra-window temperature

* $P < 0.05$ vs. pre hypercapnia, control or pre hypotension, control

† $P < 0.05$ vs. post hypercapnia, control or post hypotension, control

# $P < 0.05$ vs. pre hypercapnia, JM-1232(−) or pre hypotension, JM-1232(−)

‡ $P < 0.05$ vs. other three groups

JM-1232(−) has characteristics that are similar to midazolam and propofol.

In the present study, basal anesthesia by isoflurane was needed for stable experiment. Because the basal anesthetic state with isoflurane might affect the tone of cerebral arterioles, we cannot exclude the possibility that the effects we observed on pial arteriolar tone after JM-1232(−) administration could be modulated, at least in part, by the presence of isoflurane.

Because JM-1232(−) has cerebrovascular dilative effect, which is not so different from propofol, and little effect on hypercapnic response, it could be safely used for sedating or anesthetizing severely ill patients, including those with intracranial disorder.

Acknowledgments This research was supported by Grant-in-Aid for Scientific Research No. 21591970 and 24592294 from the Ministry of Education, Culture, Sports, Science and Technology, Japan. The authors would like to thank Enago (www.enago.jp) for the English language review.

Conflict of interest None.

References

1. Kanamitsu N, Osaki T, Itsuji Y, Yoshimura M, Tsujimoto H, Soga M. Novel water-soluble sedative-hypnotic agents: isoindolin-1-one derivatives. Chem Pharm Bull (Tokyo). 2007;55:1682–8.
2. Sneyd JR, Rigby-Jones AE, Cross M, Tominaga H, Shimizu S, Ohkura T, Grimsehl K. First human administration of MR04A3: a novel water-soluble nonbenzodiazepine sedative. Anesthesiology. 2012;116:385–95.
3. Moriyama T, Tsuneyoshi I, Kanmura Y. Effects of a novel benzodiazepine derivative, JM-1232(−), on human gastroepiploic artery in vitro. J Cardiothorac Vasc Anesth. 2011;25:72–7.
4. Iida H, Iida M, Takenaka M, Fukuoka N, Dohi S. Comparative effects of cilostazol and aspirin on the impairment of endothelium-dependent cerebral vasodilation caused by acute cigarette smoking in rats. J Thromb Thrombolysis. 2010;29:483–8.
5. Iida M, Iida H, Takenaka M, Tanabe K, Iwata K. Preventive effect of varenicline on impairment of endothelial function in cerebral vessels induced by acute smoking in rats. J Anesth. 2012;26:928–31.

6. Kumano H, Shimomura T, Furuya H, Yomosa H, Okuda T, Sakaki T, Kuro M. Effects of flumazenil during administration of midazolam on pial vessel diameter and regional cerebral blood flow in cats. Acta Anaesthesiol Scand. 1993;37:567–70.
7. Shibuya K, Ishiyama T, Ichikawa M, Sato H, Okuyama K, Sessler DI, Matsukawa T. The direct effects of propofol on pial microvessels in rabbits. J Neurosurg Anesthesiol. 2009;21:40–6.
8. Park WK, Lynch C 3rd, Johns RA. Effects of propofol and thiopental in isolated rat aorta and pulmonary artery. Anesthesiology. 1992;77:956–63.
9. Iida H, Ohata H, Iida M, Watanabe Y, Dohi S. Isoflurane and sevoflurane induce vasodilation of cerebral vessels via ATP-sensitive K + channel activation. Anesthesiology. 1998;89:954–60.
10. Forster A, Juge O, Morel D. Effects of midazolam on cerebral hemodynamics and cerebral vasomotor responsiveness to carbon dioxide. J Cereb Blood Flow Metab. 1983;3:246–9.
11. Strebel S, Kaufmann M, Guardiola PM, Schaefer HG. Cerebral vasomotor responsiveness to carbon dioxide is preserved during propofol and midazolam anesthesia in humans. Anesth Analg. 1994;78:884–8.
12. Matta BF, Mayberg TS, Lam AM. Direct cerebrovasodilatory effects of halothane, isoflurane, and desflurane during propofol-induced isoelectric electroencephalogram in humans. Anesthesiology. 1995;83:980–5.