



## EFFECTS OF MIDAZOLAM ON THE SPINAL CORD BLOOD FLOW DURING HYPOVOLEMIC SHOCK IN CATS

Tomoki Nishiyama\*

Department of Anesthesiology, Kamakura Hospital, Kanagawa, Japan.

\*Corresponding Author: Dr. Tomoki Nishiyama

Department of Anesthesiology, Kamakura Hospital, Kanagawa, Japan.

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### ABSTRACT

The purpose of this study was to investigate the changes of spinal cord blood flow by midazolam in hypovolemic shock in the cat model. Twelve male cats of 2.5 to 3.5 kg body weight were tracheotomized and ventilated to keep end-tidal carbon dioxide (CO<sub>2</sub>) tension between 30 and 35 mmHg under 1.5% isoflurane in 40% oxygen. The cats were fixed in a stereotaxic apparatus, and L1 to L5 were laminectomized to expose the spinal cord. A platinum electrode was stereotaxically inserted into the spinal cord to a depth of 1 mm at 2 mm lateral of the midline at L2. The spinal cord blood flow was measured with a hydrogen clearance method. Immediately after the intravenous administration of normal saline 5 mL including (Midazolam group, N=6) or without (Control group, N=6) midazolam 1 mg/kg, arterial blood was drawn to decrease blood pressure to the half of the control value in 5 minutes. The low blood pressure was kept for 30 minutes, and then all drawn blood was re-administered in 10 minutes. Blood pressure, heart rate, and spinal cord blood flow were measured until 30 minutes after the return of the blood. Spinal cord blood flow decreased by blood drawn in both groups but to a lesser extent in the Midazolam group during shock phase and at 30 minutes after blood return. In conclusion, during hypovolemic shock by bleeding, midazolam attenuated the decrease of the spinal cord blood flow.

**KEYWORDS:** Benzodiazepine, Midazolam, ischemia, bleeding, spinal cord, blood flow.

### 1. INTRODUCTION

Midazolam is widely used for anesthesia and sedation in the intensive care units. It has neuroprotective effects in animal experiment with cerebral ischemia<sup>[1]</sup>, and has been shown to decrease cerebral blood flow, and intracranial pressure.<sup>[2]</sup> Therefore, midazolam is useful to protect the brain from ischemic damage.

The spinal cord blood flow was recently studied to increase by midazolam when blood pressure did not change, while the spinal cord blood flow did not change when blood pressure decreased by high dose midazolam.<sup>[3]</sup> The present study focused on the spinal cord blood flow in shock. We investigated the effects of midazolam on the spinal cord blood flow in hypovolemic shock in the cat model.

### 2. MATERIALS AND METHODS

**2.1. Materials:** After obtaining approval of the protocol by institutional animal committee, 12 male cats of 2.5 to 3.5 kg in body weight (Nippon Biosupply, Tokyo, Japan) were used in this study.

**2.2. Animal preparation:** Tracheostomy was performed under 4% isoflurane anesthesia in 100% oxygen by a

mask. After tracheostomy, artificial ventilation was maintained. The femoral vein was cannulated to infuse lactated Ringer's solution at 30 mL/kg/h. Muscle relaxation was obtained with intravenous vecuronium 5 mg, followed by 0.5 mg every 30 minutes. End-tidal carbon dioxide (CO<sub>2</sub>) tension was kept between 30 and 35 mmHg under 1.5% isoflurane in 40% oxygen mixed with air. The femoral artery was cannulated to monitor blood pressure. Heart rate was monitored by electrocardiogram. Rectal temperature was monitored and was kept at 37.0 ± 0.5 °C by a heating blanket and a heating lamp.

The cats were fixed in a stereotaxic apparatus. With a midline skin incision, after local anesthesia with 1% lidocaine, L1 to L5 were laminectomized and the spinal cord was exposed. A platinum electrode (standard needle type 100 μm diameter, UHE-100, Unique Medical, Tokyo, Japan) was stereotaxically inserted into the spinal cord to a depth of 1 mm, 2mm laterally from the midline at L2. Isoflurane concentration was decreased to 0.5% during the study.

**2.3. Measurements:** The spinal cord blood flow was measured with a hydrogen clearance method.<sup>[4]</sup> The

local spinal cord blood flow (during 2 minutes following the first 30 seconds of the wash out curve) was calculated with a flow analyzer (MHG-D1, Unique Medical, Tokyo, Japan) based on the blood-tissue exchange theory by Kety and Shmidt.<sup>[5]</sup>

**2.4. Study protocol:** Immediately after the intravenous administration of normal saline 5 mL including midazolam 1 mg/kg (Midazolam group, N=6) or without it (Control group, N=6), arterial blood was gradually drawn to decrease blood pressure to the half of the control value in 5 minutes. The drawn blood was kept in a heparinized bag in the refrigerator. The low blood pressure was kept for 30 minutes, and then blood was returned in 10 minutes. Anesthetic concentration was not changed and no vasoactive agents were administered.

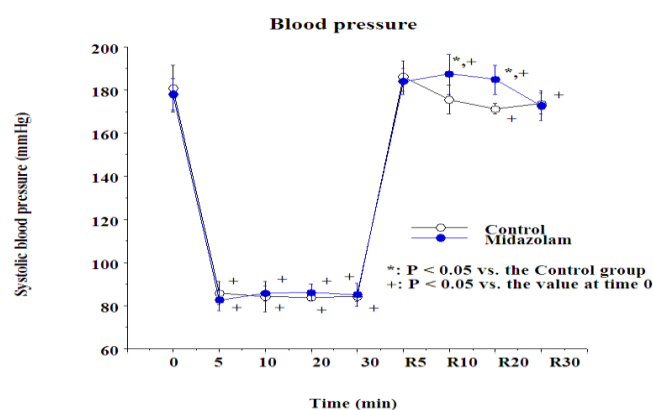
Blood pressure, heart rate, and spinal cord blood flow were measured during a period of 30 minutes after the return of the blood.

**2.5. Data analysis:** Data were expressed as mean  $\pm$  standard deviation. Power analysis was performed to detect the differences of spinal cord blood flow between

two groups with power of 0.90 and effect size of 0.3 using the G Power™ software (University of Mannheim, Germany), then 12 cats were studied. Statistical analysis was performed with repeated measures analysis of variance, followed by the Student-Newman-Keuls test.  $P < 0.05$  was considered statistically significant.

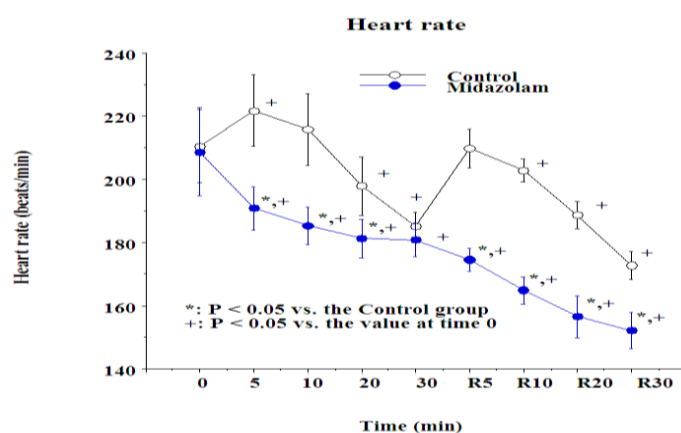
## 2. RESULTS

The volume of drawn blood was  $75 \pm 25$  mL in the Midazolam group and  $82 \pm 19$  mL in the Control group, which had no difference. End-tidal  $\text{CO}_2$  was not different between the two groups (Data are not shown.). Blood pressure decreased to about half of the control value in 5 minutes in both groups and was constant for 30 minutes. The Midazolam group had significantly higher blood pressure 10 and 20 minutes after blood return (Fig. 1). Heart rate gradually decreased in the Midazolam group, while it increased after blood drawn and after blood return transiently in the Control group. Heart rate was significantly lower in the Midazolam group than the Control group (Fig. 2). Spinal cord blood flow decreased by blood drawn in both groups but to a lesser extent in the Midazolam group during shock phase and at 30 minutes after blood return (Fig. 3).



**Figure 1. Blood pressure**

Systolic blood pressure is shown. Bars indicate standard deviation. R5, R10, R20, R30 mean 5, 10, 20, and 30 minutes after blood return, respectively.



**Figure 2. Hear rate**

Bars indicate standard deviation. R5, R10, R20, R30 mean 5, 10, 20, and 30 minutes after blood return, respectively.

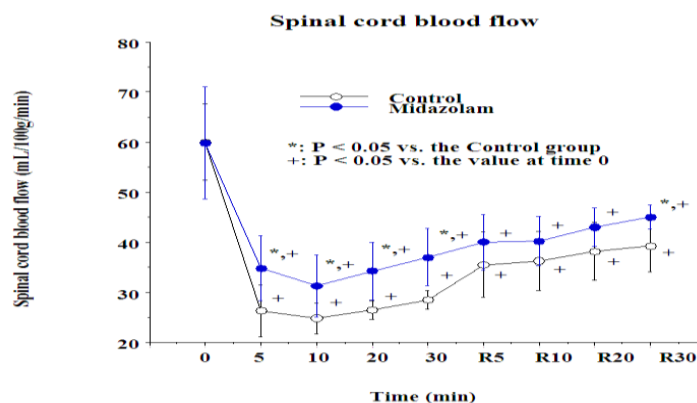


Figure 3. Spinal cord blood flow

Bars indicate standard deviation.

R5, R10, R20, R30 mean 5, 10, 20, and 30 minutes after blood return, respectively.

### 3. DISCUSSION

The results showed that midazolam 1 mg/kg administered just before hypovolemic shock attenuated the decrease of the spinal cord blood flow.

Transient increases in heart rate observed twice in the Control group might be due to sympathetic activation against sudden hypovolemia by blood drawn and hypervolemia by blood return, which could not be blocked during light isoflurane anesthesia, while in the Midazolam group, midazolam could block that response.

In the present study, the spinal cord blood flow before drug administration was about 60 mL/100g/min, which was almost the same as in a previous study investigating spinal cord blood flow in cats under isoflurane anesthesia<sup>[3]</sup> and in the study by Landau et al.<sup>[6]</sup>

Many factors influence spinal cord blood flow. There was essentially no difference found in the autoregulatory capacities between spinal cord blood flow and cerebral blood flow. Spinal cord blood flow does not change with mean blood pressure between 50 and 135 mmHg as the result of an autoregulation,<sup>[7]</sup> or with arterial oxygen pressure (PaO<sub>2</sub>) higher than 40 mmHg<sup>[8]</sup>

Spinal cord blood flow increases as arterial CO<sub>2</sub> pressure (PaCO<sub>2</sub>) increases.<sup>[9]</sup> The increase of PaCO<sub>2</sub> by 1 mmHg increases spinal cord blood flow by 0.15 to 1.17 mL/100 g/min. without autoregulation.<sup>[10]</sup>

Even a high dose (10 mg/kg in cats) of midazolam did not affect the responsiveness of the vessels to CO<sub>2</sub> and regional cerebral blood flow.<sup>[11]</sup> In our study, end-tidal CO<sub>2</sub> was kept in the range between 30 and 35 mmHg and no significant difference was found between the two groups, therefore, the effects of CO<sub>2</sub> could be negligible.

Spinal cord blood flow might change similarly to cerebral blood flow. Blood flow thresholds and limits for the development of neurologic deficits of the spinal cord are comparable to those of the brain.<sup>[12]</sup> Interruption of spinal cord blood supply for 15 to 20

minutes may be tolerated with complete recovery if regional spinal cord blood flow remains more than 50%.<sup>[13]</sup> The hypovolemic shock model in the present study showed spinal cord blood flow less than 50% of the control flow, therefore, the Control group might have damage of the spinal cord. The Midazolam group could keep the spinal cord blood flow more than 50% of the control flow, which might protect the spinal cord from ischemic damage.

Midazolam was reported to decrease cerebral blood flow whether blood pressure decreased<sup>[2]</sup> or did not change.<sup>[14]</sup> However, spinal cord blood flow increased by midazolam in our previous study<sup>[3]</sup> when blood pressure did not change, and spinal cord blood flow did not change when blood pressure decreased. Therefore, the effects of midazolam might be different between cerebral blood flow and spinal cord blood flow.

The present results showed higher spinal cord blood flow in the Midazolam group than the Control group during hypovolemic shock. Blood pressure during shock was not different between the two groups. Therefore, the higher spinal cord blood flow in the Midazolam group did not depend on systemic blood pressure.

Blood pressure was higher after return of the blood and heart rate was lower during and after shock in the Midazolam group compared to those in the Control group. These might be due to the effects of midazolam to decrease stress response induced by hypovolemic shock. Both these general hemodynamic effects and the effects on the spinal cord blood flow might be beneficial at shock during anesthesia or sedation. As whether these effects of midazolam were useful in shock was not confirmed from the present study because histopathological spinal cord damage, outcome and behavioral side effects were not studied.

The mechanism to keep spinal cord blood flow higher with midazolam is not clear. Further study with denervation or with gamma-aminobutyric acid (GABA) antagonist is necessary.

Midazolam diminished increases in expression of cerebral endothelial intercellular adhesion molecule-1 and P-selectin expression following hypoxia-reoxygenation.<sup>[15]</sup> Midazolam attenuated neurological deficits and reduced infarct and edema volumes, and decreased excitatory amino acids accumulation in focal cerebral ischemia-reperfusion rat model.<sup>[16]</sup> Then, midazolam had a strong neuroprotective effect.<sup>[17]</sup> We could expect the same mechanisms for midazolam to protect spinal cord from ischemic damage, while further histopathological and behavioral studies are necessary to confirm the usefulness of midazolam in ischemic spinal cord damage.

#### 4. CONCLUSIONS

During hypovolemic shock by bleeding, midazolam attenuated the decrease of the spinal cord blood flow.

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