Kazuyoshi Hirota MD,
Noriaki Ohtomo MD,
Yoshio Hashimoto MD,
Tsuyoshi Kudo MD,
Hironori Ishihara MD,
Akitomo Matsuki MD

Midazolam reverses histamine-induced bronchoconstriction in dogs

Purpose: Midazolam has been used clinically as a sedative and as an anaesthetic induction agent. However, the bronchodilating effects of midazolam have not been comprehensively evaluated. We sought to determine relaxant effects of midazolam on the airway.

Methods: After our Animal Care Committee approved the study, eight mongrel dogs were anaesthetized with 30 mg·kg⁻¹ pentobarbitone iv, and were paralysed with 200 µg·kg⁻¹·hr⁻¹ pancuronium. The trachea was intubated with an endotracheal tube (ID 7 mm) that had a second lumen for insertion of a superfine fiberoptic bronchoscope (OD 2.2 mm) to measure the bronchial cross-sectional area (BCA) continuously. The tip of the bronchoscope was placed at the level of the second or third bronchial bifurcation of the right bronchus. A videoprinter printed the BCA which was then measured with a NIH Image program. Bronchoconstriction was produced with histamine (H) 10 µg·kg⁻¹ followed by 500 µg·kg⁻¹·hr⁻¹. Thirty minutes later, 0 [saline], 0.01, 0.1 and 1.0 mg·kg⁻¹ midazolam and 25 µg·kg⁻¹ flumazenil were given. The BCA was assessed before (basal area) and 30 min after the start of H infusion, and was also measured five minutes after each midazolam and flumazenil iv. At the same time, arterial blood was sampled for plasma catecholamine measurement.

Results: Histamine infusion decreased BCA to 49.7 ± 17.3% of basal BCA. More than 0.1 mg·kg⁻¹ midazolam increased BCA up to 71.7 ± 15.3% of the basal (1.0 mg·kg⁻¹) ($P < 0.01$). Plasma adrenaline concentration was decreased from 6.9 ± 3.8 to 3.7 ± 1.9 ng·ml⁻¹ by 1.0 mg·kg⁻¹ midazolam ($P < 0.05$). Flumazenil did not antagonize the relaxant effect of midazolam but reversed the inhibitory effect of midazolam on histamine-induced adrenaline release.

Conclusion: Midazolam has a spasmolytic effect on constricted airways but this bronchodilatation was not reversed by flumazenil.

Objectif : En clinique, on utilise le midazolam comme sédatif et comme agent d'induction de l'anesthésie. Cependant, les effets bronchodilatateurs du midazolam n'ont jamais fait l'objet d'une évaluation complète. Nous avons recherché les effets relaxants du midazolam sur les voies aériennes.

Méthodes : Après l'approbation du comité d'expérimentation animale, huit chiens de race commune ont été anesthésiés avec 30 mg·kg⁻¹ de pentobarbital iv et paralysés avec 200 µg·kg⁻¹·h⁻¹ de pancuronium. La trachée a été intubée avec un tube endotrachéal (DI 7 mm) avec une deuxième lumière pour l'insertion d'un bronchofibroscope extra-fin (DE 2,2 mm) pour la mesure de la surface transversale bronchique (STB) en continu. L'extrémité du bronchoscope a été placée au niveau de la deuxième ou troisième bifurcation de la bronche droite. Une imprimante vidéo imprimait la mesure de la STB avec un programme d'imagerie du NIH. La bronchoconstriction était provoquée avec de l'histamine (H) 10 µg·kg⁻¹ suivie d'une perfusion de 500 µg·kg⁻¹·h⁻¹. Trente minutes plus tard, on administrait 0 mg, 0,01 mg, 0,1 mg et 1,0 mg·kg⁻¹ de midazolam et 25 µg·kg⁻¹ de flumazénil. La STB était évaluée avant (surface initiale) et 30 min après le début de la perfusion H et une autre fois cinq min après chaque injection iv de midazolam et de flumazénil. Au même moment, un échantillon de sang était prélevé pour le titrage des catécholamines.

Résultats : La perfusion d'histamine a réduit la STB de 49 ± 17,3% de la valeur initiale. Une dose de plus de 0,1 mg·kg⁻¹ de midazolam augmentait la STB jusqu'à 71,7 ± 15,3% de la valeur initiale (1 mg·kg⁻¹, $P < 0,01$). Le midazolam 1,0 mg·kg⁻¹ diminuait la concentration plasmatique d'adrénaline de 6,9 ± 3,8 à 3,7 ± 1,8 ng·ml⁻¹ ($P < 0,05$). Le flumazénil n'a pas inhibé la relaxation produite par le midazolam mais a aboli l'effet inhibiteur du midazolam sur la libération d'adrénaline induite par l'histamine.

Conclusion : Le midazolam a un effet spasmolytique sur la bronchoconstriction mais cet effet n'est pas aboli par le flumazénil.

From the Department of Anesthesiology, University of Hirosaki School of Medicine, Hirosaki 036, Japan.

Address correspondence to: Dr. K. Hirota; Phone: +81-172-33-5111; Fax: +81-172-39-5112.

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BENZODIAZEPINES are widely used in clinical practice because of their anxiolytic, muscle relaxant, anticonvulsant and sedative effects. Midazolam is used as an anaesthetic induction agent for critically ill patients in the operating room and for sedation in the Intensive Care Units because it does not have potent depressive cardiovascular effects.¹ Several *in vitro* studies indicate that midazolam has a spasmolytic effect on constricted airway smooth muscle. However, *in vivo* its effect has not been studied.²⁻⁴

Airway calibre is normally assessed indirectly by measuring airway resistance or compliance, which can be strongly influenced by airway secretions.⁵ However, there are several reports of the relative insensitivity of indirect measurements of airway calibre.⁶⁻⁸ We have developed a portable direct method of quantifying bronchial calibre using a superfine fiberoptic bronchoscope.⁹⁻¹¹ Also, we have shown that the direct visualization method is more specific in the assessment of airway calibre. When assessed by bronchial cross-sectional area (BCA), adrenaline was shown to reverse histamine-induced bronchoconstriction completely, although dynamic pulmonary compliance (C_{dyn}) and airway resistance (Raw) had not returned to pre-histamine values, although there were correlations between BCA and C_{dyn} ($r = 0.720$, $P < 0.0001$) and BCA and Raw ($r = 0.727$, $P < 0.0001$).¹¹

In the present study, BCA was employed to evaluate the *in vivo* spasmolytic effect of midazolam and the antagonistic effect of flumazenil on histamine-induced bronchoconstriction in dogs.

Methods

The study protocol was approved by the Animal Care and Use Committee in the University of Hirosaki School of Medicine. Eight mongrel dogs (8–12 kg) were anaesthetized with 30 mg·kg⁻¹ pentobarbitone *iv* and paralysed by continuous infusion of 200 µg·kg⁻¹·hr⁻¹ pancuronium. If anaesthesia was not deep enough during experiment, 5 mg·kg⁻¹ pentobarbitone was given although the induction dose could maintain anaesthesia adequately during the experiments.¹² The tracheas were intubated with endotracheal tubes (ID: 7.0 mm, Univent tube, Fuji System, Tokyo) that had a second lumen usable for a bronchial blocker. The lungs were ventilated with oxygen and the end-tidal CO₂ was maintained at 4.0–4.5%. A femoral artery and vein were cannulated to monitor arterial blood pressure and to insert a Swan-Ganz catheter (Baxter Health Co, Tokyo).

Imaging and analysis of airway

A superfine fiberoptic bronchoscope (OD 2.2 mm: AF type 22A, Olympus, Tokyo) was inserted through the second lumen of the endotracheal tube placing the

tip between the second and third bronchial bifurcation of the right bronchus to monitor continuously the bronchial cross-sectional area (BCA) at the third bifurcation. Previously, we have shown that insertion of the bronchoscope did not change anaesthesia depth as plasma concentrations of noradrenaline and adrenaline were not altered between 30 min (138 ± 45 and 162 ± 18 pg·ml⁻¹) and 60 min (188 ± 48 and 136 ± 18 pg·ml⁻¹, respectively) after bronchoscope insertion. Also, the tip of bronchoscope could be well fixed as BCA did not change during time.⁹ The BCA data after expiration and before inspiration were printed out with a videoprinter (VY-170, Hitachi, Tokyo). Subsequently, analysis of the BCA was performed on a Macintosh computer using the public domain NIH Image program.^a

Protocol (Figure 1)

Basal BCA measurements were made prior to beginning histamine administration. Bronchoconstriction was produced by 10 µg·kg⁻¹ histamine *iv* followed by a continuous infusion of 500 µg·kg⁻¹·hr⁻¹ histamine via the pulmonary arterial line of Swan-Ganz catheter until the end of the experiment. In a preliminary study, we found that a stable BCA could be obtained 20–30 min after the start of the histamine infusion. Systolic blood pressure was maintained >80 mmHg with fluid (lactate Ringer solution: 50 ml·kg⁻¹) and with phenylephrine infusion (initially 0.5–2.0 µg·kg⁻¹·min⁻¹) titrated according to systolic blood pressure. Thirty minutes after the start of the histamine infusion, 0 [saline], 0.01, 0.1, 1.0 mg·kg⁻¹ midazolam and 25 µg·kg⁻¹ flumazenil (specific central benzodiazepine antagonist) were given in this order at five minute intervals. The BCA was measured five minutes after each dose of midazolam and flumazenil. The BCA is presented as % of basal BCA, respectively: % BCA = (measured BCA ÷ basal BCA) × 100.

Experiments were terminated if: a) airway secretion caused by histamine infusion covered the tip of the bronchoscope and BCA could not be measured, or b) histamine infusion did not decrease BCA by > 30%.

Catecholamines assay

Arterial blood (6 ml) was collected through the femoral artery simultaneously with BCA measurements to determine plasma adrenaline and noradrenaline concentrations. Blood samples were immediately centrifuged to separate plasma which was then frozen at -70°C until the assay. Plasma concentrations of

^a Wayne Rasband, US National Institutes of Health, available from Internet by anonymous ftp from [zipper.nimh.nih.gov](ftp://zipper.nimh.nih.gov) or on floppy disk from NTIS, 5285 Port Royal RD., Springfield, VA 22161, part number PB93-504868).

Drug administration	H infusion ($10 \mu\text{g}\cdot\text{kg}^{-1} + 500 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) →										
	↓	Saline (M 0 mg·kg ⁻¹)	M 0.01 mg·kg ⁻¹	M 0.1 mg·kg ⁻¹	M 1.0 mg·kg ⁻¹	FLM 25 $\mu\text{g}\cdot\text{kg}^{-1}$					
Time (min)	0	30	35	40	45	50	55	60	65	70	75
BCA measurement	(+)	(+)	(+)		(+)		(+)		(+)		(+)
Catecholamine assay		(+)	(+)		(+)		(+)		(+)		(+)

FIGURE 1 Order of study drug administered. BCA: bronchial cross-sectional area, H: histamine, M 0.01: 0.01 mg·kg⁻¹ midazolam, FLM: 25 $\mu\text{g}\cdot\text{kg}^{-1}$ flumazenil.

adrenaline and noradrenaline were determined with gas chromatography mass spectrometry.¹³ The assay coefficient of variation was 8.4% for adrenaline and 11.3% for noradrenaline.

Statistical analysis

All data are expressed as mean $\pm$ SD. Data were analysed using repeated measures ANOVA followed by Fisher's protected least significant difference test using Stat View 2.0 on Macintosh computer. $P < 0.05$ was considered significant.

Results

No experiment was terminated by airway secretion and ineffective histamine infusion. Histamine decreased BCA to $49.7 \pm 6.1\%$ of basal BCA. Doses $> 0.1 \text{ mg}\cdot\text{kg}^{-1}$ midazolam increased % BCA (0.1 and $1.0 \text{ mg}\cdot\text{kg}^{-1}$ midazolam: $67.9 \pm 15.3\%$, $P < 0.01$ and $71.7 \pm 15.3\%$, $P < 0.01$, respectively) (Figure 2). Flumazenil did not antagonize midazolam-induced relaxation (Figure 2).

Plasma adrenaline concentration decreased from 6.9 ± 3.8 to $3.7 \pm 1.9 \text{ ng}\cdot\text{ml}^{-1}$ ($P < 0.05$) after $1.0 \text{ mg}\cdot\text{kg}^{-1}$ midazolam. Flumazenil increased the reduced adrenaline level after midazolam ($1.0 \text{ mg}\cdot\text{kg}^{-1}$) to $6.3 \pm 2.9 \text{ ng}\cdot\text{ml}^{-1}$ ($P < 0.05$, Figure 3). Although plasma noradrenaline concentrations changed in a similar direction as those of adrenaline; decrease from 495 ± 164 to $412 \pm 208 \text{ pg}\cdot\text{ml}^{-1}$ after $1.0 \text{ mg}\cdot\text{kg}^{-1}$ midazolam and increase to $488 \pm 244 \text{ pg}\cdot\text{ml}^{-1}$ after flumazenil, the changes did not reach statistical significance (Figure 3).

Discussion

In the present study, we found that midazolam (doses $> 0.1 \text{ mg}\cdot\text{kg}^{-1}$) relaxed *in vivo* histamine-induced bronchoconstriction. Recent studies²⁻⁴ have also demonstrated that midazolam has bronchodilating effects *in vitro*. However, the mechanism by which midazolam exerts bronchodilating effects has not been clearly defined. It has been reported that GABA inhibits postganglionic cholinergic neurotransmission¹⁵ and, thus, the spasmolytic effect might be due to GABA receptor activation by midazolam bound to benzodiazepine

FIGURE 2 Spasmolytic effect of midazolam (M) on histamine (H)-induced bronchoconstriction assessed by changes in bronchial cross-sectional area (BCA).

Pre: before start of H infusion. H30': 30 min after the start of H infusion, M 0.01: $0.01 \text{ mg}\cdot\text{kg}^{-1}$ midazolam, FLM $25 \mu\text{g}\cdot\text{kg}^{-1}$ flumazenil, * $P < 0.01$ compared with H30'. All values are expressed as mean $\pm$ SD.

FIGURE 3 Effect of midazolam (M) and flumazenil (FLM) on plasma adrenaline and noradrenaline concentrations.

H30': 30 min after the start of H infusion, M 0.01: $0.01 \text{ mg}\cdot\text{kg}^{-1}$ midazolam, FLM: $25 \mu\text{g}\cdot\text{kg}^{-1}$ flumazenil, * $P < 0.05$ compared with H30', † $P < 0.05$ compared with FLM. All values are expressed as mean $\pm$ SD.

binding sites at the GABA receptor. However, we observed that flumazenil, a central benzodiazepine receptor antagonist, did not reverse the midazolam-relaxation. Several *in vitro* studies^{2,4,16} have also shown that the relaxation is not mediated through central benzodiazepine receptors because it is not reversed by flumazenil. Raeburn *et al.*¹⁶ found peripheral benzodiazepine binding sites in airway smooth muscle by radioligand ($[^3\text{H}]\text{Ro5-4684}$) and showed that Ro5-4684, a peripheral benzodiazepine agonist, produces spasmolytic effect on airway smooth muscle. However, they also showed that midazolam did not displace $[^3\text{H}]\text{Ro5-4684}$ bound to benzodiazepine binding sites in airway smooth muscle, and that PK11195, peripheral benzodiazepine receptor antagonist could not reverse midazolam-induced spasmolytic effect. Other investigators^{2,4} have observed no effect of PK11195 on midazolam relaxation. Therefore, it is unlikely that midazolam produces spasmolytic effects via central or peripheral benzodiazepine receptor activation.

Yoshimura *et al.*⁴ found that midazolam relaxed K^+ - and carbachol-induced contraction of airway smooth muscle in accordance with a decrease in intracellular calcium by inhibition of the influx of extracellular Ca^{2+} , and that central and peripheral benzodiazepine antagonist could not reverse the relaxation. Therefore, voltage sensitive Ca^{2+} channels may be involved in the mechanism.

Previously, we found that direct visualization using a superfine fiberoptic bronchoscope was more sensitive than dynamic pulmonary compliance and airway resistance in assessing airway calibre.¹⁰ However, one problem of the endoscopic technique was that when airway secretion, caused by histamine infusion, covered the tip of the bronchoscope the measurements cannot be continued. However, in the present study we did not experience this situation.

Gal and Suratt¹⁴ reported that tracheal intubation produced bronchoconstriction, but they did not show whether the irritant effect was tolerable 30 min after intubation. We previously assessed BCA and plasma concentrations of catecholamines at 30 and 60 min after bronchoscope placement.⁹ Neither BCA nor plasma concentrations of adrenaline or noradrenaline ($< 200 \text{ pg}\cdot\text{ml}^{-1}$) changed. Therefore, we suggest that pentobarbitone anaesthesia, used in the present study, was sufficient to prevent the irritant effect.

In the present study flumazenil reversed the decrease in plasma adrenaline concentration produced by midazolam. Other investigators^{17,18} have also suggested that midazolam has sympatholytic effects that can be antagonized by flumazenil. Alpha sympathetic blockers¹⁹ produce bronchodilation, and epidural

block²⁰ has been reported to be effective in the treatment of asthma, although sympatholytic actions generally worsen bronchoconstriction. However, the dose of flumazenil that reversed midazolam sympatholysis did not antagonize the midazolam bronchodilating effect. Therefore, sympatholysis is unlikely to have been involved in the mechanism of bronchodilatation.

The present study suggests that midazolam is not an effective therapeutic agent in status asthmaticus because even in doses of $1.0 \text{ mg}\cdot\text{kg}^{-1}$ midazolam reversed histamine-induced bronchoconstriction by only 40%. However, it suggests that midazolam could be used safely as a sedative for such patients during mechanical ventilatory support without worsening the bronchoconstriction.

In conclusion, employing a direct visualization method using superfine fiberoptic bronchoscope, we found that midazolam has a spasmolytic effect on histamine-induced bronchoconstriction, an effect that was not reversed by flumazenil.

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