Laboratory Investigations

Intrathecal midazolam reduces isoflurane MAC and increases the apnoeic threshold in rats

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The purpose of this study was to examine the anaesthetic requirement of intrathecal midazolam in a dose-response fashion in isoflurane-anaesthetized, tracheostomized rats, and to evaluate the apnoeic threshold after each intrathecal midazolam dose. Intrathecal midazolam, 5, 10, 20, and 30 μg, was administered to 25 anaesthetized tracheotomized rats. Isoflurane MAC was determined by the tail-clamp method. The effect of intrathecal midazolam on the apnoeic threshold was evaluated, and light and electron microscopy studies were performed on cervical, thoracic and lumbar sections of the spinal cord to investigate possible midazolam-induced neurotoxic effects. Intrathecal midazolam 5, 10, 20 and 30 μg decreased isoflurane MAC by 16%, 31%, 42%, and 53% respectively ($P < 0.05$). The apnoeic threshold was increased by midazolam 5 μg (from a $PaCO_2$ of 4.25 ± 0.55 to 5.28 ± 0.76 kPa, $P < 0.05$) when compared with baseline values, but not further by additional doses. Light and electron microscopy studies on sections taken from the spinal cord of four animals did not show any morphological changes suggestive of midazolam-induced neurotoxicity when compared with similar preparations obtained from controls. These data suggest that intrathecal midazolam possesses dose-dependent antinociceptive properties which, associated with the ceiling effect of the apnoeic threshold obtained at the lowest midazolam dose and the lack of neurotoxic effects, may potentiate inhalational anaesthesia without producing marked respiratory depression.

Ce travail vise à déterminer la relation dose-effet du midazolam intra-thécal chez le rat trachéotomisé, anesthésié à l'isoflurane et de rechercher le seuil apnéique après chaque dose intrathécale de midazolam. Des doses de midazolam intra-thécal de 5, 10, 20 et 30 μg sont administrées à 25 rats anesthésiés et trachéotomisés. Le MAC de l'isoflurane est déterminé par la méthode de clampage de la queue. L'effet du midazolam sur le seuil apnéique est évalué et des études de microscopie optique et électronique réalisées sur des sections de moelle cervicale, thoracique et lombaire pour vérifier la neurotoxicité du midazolam. Les doses de midazolam intra-thécal de 5, 10, 20, et 30 μg réduisent le MAC de l'isoflurane de 16%, 31%, 42% et 53% respectivement ($P < 0,05$). Le seuil apnéique est augmenté par le midazolam 5 μg (d'une $PaCO_2$ de 4,25 ± 0,55 à 5,28 ± 0.76 kPa, $P < 0,05$) comparativement aux valeurs de base, sans autres modifications après des doses additionnelles. Les coupes étudiées à la microscopie optique et électronique n'ont pas montré de changements morphologiques évocateurs de neurotoxicité lorsqu'elles sont comparées à des spécimens identiques prélevés sur les contrôles. Ces données suggèrent que le midazolam intra-thécal possède des propriétés antinociceptives qui, associées au plafonnement des effets sur

Key words

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le seuil apnéique de la dose inférieure de midazolam et à l'absence de neurotoxicité, pourraient potentialiser l'anesthésie inhalatoire sans produire de dépression respiratoire notable.

Midazolam, a water-soluble benzodiazepine, is commonly used as an adjunct to general anaesthesia and has been shown to decrease anaesthetic requirements after intravenous administration in both animals[1] and humans.[2] The intrathecal use of opioids produces selective spinal analgesia but is associated with serious problems such as respiratory depression and tolerance.[3] Spinal agents that offer optimal antinociceptive characteristics without adverse effects may represent an alternative to intrathecal opioids. The antinociceptive properties of midazolam after intrathecal administration have recently been reported in animals,[4–7] and its therapeutic effect evaluated in patients with chronic back pain,[8,9] despite evidence of midazolam-induced neurotoxicity in one animal model.[10]

The purpose of the present study was to examine the effect of intrathecal midazolam on isoflurane MAC in a dose-response fashion in isoflurane-anaesthetized, tracheotomized rats. Since the respiratory effects of intrathecal midazolam remain unknown, the apnoeic threshold after each intrathecal dose was also evaluated. Finally, both light and electron microscopy studies were carried out on transverse sections of the cervical, thoracic, and lumbar spine to evaluate the possible neurotoxic effects of midazolam.

Methods

After institutional approval 25 male Sprague-Dawley rats weighing 350–400 g were anaesthetized with isoflurane by face mask to permit dissection of neck tissues and percutaneous insertion through a 20-G needle of a 10 cm PE-10 polyurethrane-guided catheter through the atlanto-occipital membrane into the intrathecal space. The catheter was advanced 8 cm caudally to position its tip at the lumbar level of the spinal cord, and secured to the subcutaneous tissue. The rats were then allowed to recover from anaesthesia. Animals with obvious neurological damage were excluded from the study. Two days later, the animals were tracheotomized under isoflurane anaesthesia, a PE-50 catheter inserted into the carotid artery for continuous haemodynamic surveillance and blood gas analyses, and the permeability of the intrathecal catheter verified by the presence of a positive CSF reflux. Mechanical ventilation was obtained with a Harvard rodent respirator, and was adjusted to maintain $PaCO_2$ at 3.5–4.0 kPa. After an observation period of one hour, baseline isoflurane MAC was determined by the tail-clamp method as previously described.[1] The MAC was defined as that end-tidal concentration to the nearest 0.1% midway between the end-tidal concentration of isoflurane at which animals did or did not move in response to the applied stimulus. Isoflurane was then discontinued and the animal was hyperventilated until the end-expiratory isoflurane concentration reached was 0.1%. The Harvard ventilator was then stopped, a 1 $L \cdot min^{-1}$ fresh flow of 100% oxygen was given and the $PaCO_2$ at which the animal started breathing spontaneously, i.e., the apnoeic threshold, was obtained with a simultaneous analysis of arterial blood gases. The animals were not restrained with any mechanical means when the isoflurane was turned off to allow breathing and determination of the apnoeic threshold. Blood gases were obtained in the brief time between recovery of spontaneous respiration and movement. If movement did occur during this time, the animal was restrained manually until the blood gas was obtained and the animal was reanaesthetized with isoflurane.

Midazolam 5 μg or 10 μg diluted in 10 μl saline was then administered intrathecally to each animal. After determination of both isoflurane MAC and the apnoeic threshold, additional doses of midazolam 5 μg and 10 μg in 10 μl saline were administered sequentially to obtain cumulative midazolam doses of 10 μg, 20 μg, and 30 μg. Seven animals received 5 and 10 μg of midazolam, three animals received 5, 10 and 20 μg, three animals 10, 20, and 30 μg, and six animals 20 and 30 μg. No animal received more than three doses of midazolam since the duration of the experiment was not to exceed the effect of intrathecal midazolam which is about 90–120 min.[11] Isoflurane MAC and apnoeic thresholds were determined 30 min after each dose. At the end of the experiment the animals were awakened and movement of all four limbs was verified to test for gross neurological damage. Four rats were then reanaesthetized with isoflurane and perfused with a 4% paraformaldehyde phophate buffered solution for five minutes. The spinal cords of the four treated animals and of two control animals were then dissected and fixed in 4% paraformaldehyde buffered solution for 12 hr. Transversal sections of the cervical, thoracic, and lumbar segments were embedded in metacrylate for light microscopy and in Epon resin for electron microscopy. All the metacrylate-embedded specimens, including the ventral and dorsal roots, were sectioned at 3 μm and stained with haematoxylin-eosin. In addition, semithin sections for electron microscopy studies were cut at 1.5 μm and stained with the Toluidine blue/Azur II method. Thin sections were treated with the uranyl acetate/lead citrate method. A total of three slides were prepared from each segment of each animal for the light microscopy studies. In addition, two semithin slides from each segment of each animal were prepared, from which two preparations per segment were obtained

TABLE Isoflurane MAC (vol%) and $PaCO_2$ (kPa; apnoeic threshold) obtained after intrathecal administration of midazolam 5 μg, 20 μg and 30 μg. n = number of animals in each group

	Control ($n = 19$)	*5 μg* ($n = 10$)	*10 μg* ($n = 13$)	*20 μg* ($n = 12$)	*30 μg* ($n = 9$)
MAC	1.53 ± 0.16	1.28 ± 0.10*	1.06 ± 0.13*†	0.89 ± 0.17*†	0.76 ± 0.09*†
$PaCO_2$	4.25 ± 0.55	5.28 ± 0.76*	5.57 ± 0.59*	5.54 ± 0.46*	6.10 ± 1.04*

*$P < 0.05$ compared with control.
†Compared with the preceding doses.

for the electron microscopy studies. The light and electron microscopy preparations were read by a staff neuropathologist who was blinded to the treatments that the animals underwent.

Rectal temperatures, mean arterial blood pressure, and mean and peak intratracheal pressures were continuously recorded, as well as end-tidal isoflurane and CO_2 concentrations (Capnomac, AVL-Datex). Statistical analysis included t test and ANOVA with repeated measures as required, with $P < 0.05$ considered significant.

Results

Of the 25 animals in which an intrathecal catheter was placed, six presented gross neurological deficits and were excluded from the study. The remaining 19 rats were therefore included in the study and were administered intrathecal midazolam. Incremental increases in midazolam intrathecal doses produced progressive reductions in isoflurane MAC, with a maximum isoflurane MAC reduction of 50% at the highest dose (30 μg; Table).

Examination of the haemodynamic data after intrathecal administration of midazolam revealed no change in mean arterial pressure compared with that measured in the absence of midazolam (98 ± 29 mmHg vs 99 ± 32 mmHg, respectively; $P > 0.05$). There was also no difference in mean arterial pressure upon application of the tail-clamp when there was movement versus when movement did not occur (103 ± 29 mmHg vs 114 ± 30 mmHg respectively; $P > 0.05$).

The apnoeic threshold was increased by midazolam 5 μg (from a $PaCO_2$ of 4.25 ± 0.55 to 5.28 ± 0.76 kPa, $P < 0.05$; Table). Additional doses of midazolam did not further increase the apnoeic threshold.

All 19 animals were awakened after the experiment and none presented any gross neurological damage. They were then sacrificed. Examination of the spinal cord sections in four of them confirmed proper positioning of the catheters at the lumbar level. Neither light nor electron microscopy revealed any histological or ultrastructural differences between the four treated animals and the two controls. The myelin sheets and axons of the spinal tracts and roots, as well as the neurons of the anterior and posterior horns were all normal.

Discussion

Although benzodiazepines are not normally considered to be analgesics, intravenous midazolam decreased anaesthetic requirement in enflurane-anaesthetized dogs,[1] and in humans.[2] In the present study midazolam produced a dose-dependent reduction in isoflurane MAC ranging from 15% at the lowest intrathecal dose (5 μg) to 53% at the greatest (30 μg). These results support the hypothesis that midazolam possesses antinociceptive properties when administered intrathecally in the rat and suggest that some of the spinal benzodiazepine receptor sites are associated with dorsal horn systems since anaesthetic-induced unresponsiveness to noxious stimuli measured by MAC testing does not depend on cortical or forebrain structures in the rat.[12] These systems are thought to result from the interaction of the benzodiazepine receptor on the GABA-benzodiazepine-ionophore complex. In addition to the probable antinociceptive effect action of intrathecal midazolam on dorsal horn benzodiazepine receptors, it is possible that the reduction of isoflurane MAC could have been due in part to actions on the motor systems as demonstrated by Yanez *et al.*[11] Though motor dysfunction was clearly established at doses greater than 60 μg,[11] lower doses such as those chosen in the present study may have produced some alteration in the motor system which could have contributed the observed decrease in isoflurane MAC. Wang *et al.*[13] recently showed that in contrast to an opioid (fentanyl) which decreased C nociceptive reflexes, midazolam caused a much greater depression of A delta responses. Since Yaksh *et al.*[14] suggested that labour pain maybe mediated by the A delta fibres, and in the absence of any neurotoxic effect, midazolam may be an interesting alternative to or an addition to epidural or spinal opioids in obstetrics.

The antinociceptive properties of intrathecal midazolam have been reported using both short-,[7,11] and long-[4] lasting painful stimuli. These observations which suggested that intrathecal midazolam may be useful in the treatment of somatic pain have recently been confirmed in human studies,[8,9] despite the fact that some uncertainty remains as to the absence or presence of any midazolam-induced neurotoxicity. Though Serrao *et al.*[15] were un-

able to demonstrate any neurotoxic effects after intrathecal administration of midazolam and Madsen *et al.*[16] after epidural midazolam, Malinovsky *et al.*[10] reported neurotoxicity in two out of nine animals after intrathecal administration of midazolam. It is important to note that Malinovsky *et al.*[10] used 10% hydrochloric acid as the midazolam vehicle whereas neither Serrao *et al.*[15] nor Madsen *et al.*[16] did. In the present study midazolam 5 μg or 10 μg were diluted in 10 μg saline and no neurotoxic damage due to midazolam was observed under both light and electron microscopy when compared with untreated controls.

The apnoeic threshold was increased by the lowest midazolam dose (5 μg = 0.015 mg · kg^{-1}) but not any further when additional doses of midazolam were administered, i.e., demonstrating a ceiling effect. Further investigations with much greater intrathecal midazolam doses are required to confirm this finding, since the highest midazolam dose did produce a slight increase in the apnoeic threshold. The ceiling effect of the apnoeic threshold, as the ceiling effect to the anaesthetic efficacy of intravenous midazolam,[1] may be explained by the capacity of the GABA-ergic neurons to modulate respiratory neural activity down to a certain basal level only. Beggren *et al.* have shown in human volunteers that the changes in respiratory pattern including changes in $PaCO_2$ induced by midazolam were caused mainly by the first *iv* dose (0.05 mg · kg^{-1}) and not by subsequent doses.[17] Single-dose studies had previously demonstrated that *iv* midazolam depressed respiration when using a CO_2 rebreathing technique or mouth occlusion pressure[18] and non-invasive techniques.[19] All animals received a 1 L · min^{-1} fresh flow of 100% oxygen during the determination of the apnoeic threshold. The average PaO_2 at which the animals resumed spontaneous ventilation was 35 ± 12 kPa which would indicate that a hypoxic drive was probably not relevant in this situation, even if sedative doses of midazolam have been shown to depress hypoxic ventilatory responses in humans.[20] In addition to the effect of intrathecal midazolam on the dorsal horn benzodiazepine receptors, and the possible action on the motor systems, a supraspinal action is likely in order to explain the increased apnoeic threshold. The ceiling effect may have been due to minimum amount of midazolam reaching these supraspinal sites. Greater doses of intrathecal midazolam would be necessary to confirm the presence of a ceiling effect or to demonstrate a continuous dose-related effect as suggested by the trend of the data.

The midazolam doses were well tolerated haemodynamically by the animals. No animal required haemodynamic support any time. Pooled data for changes in mean arterial pressure with tail-clamp application that produced movement versus those that were produced when movement did not occur showed no statistically significant difference. This suggests once again that the degree of haemodynamic response would not be predictive of somatic (i.e., movement) responses,[21] and could not be relied upon as an indicator of inadequate isoflurane-midazolam anaesthesia.

In conclusion, the present study demonstrated that intrathecal midazolam (5–30 μg) reduced isoflurane MAC in a dose-dependent fashion confirming its antinociceptive properties. This characteristic associated with the ceiling effect of the apnoeic threshold obtained at the lowest dose and the lack of neurotoxicity suggests that intrathecal midazolam may potentiate inhalational anaesthesia without producing marked respiratory depression, as well as offer an alternative or adjunct to spinal opioids.

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