Anesthesiology
75:91–97, 1991

Ketamine and Midazolam Neurotoxicity in the Rabbit

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Ketamine and midazolam can produce analgesia following intrathecal administration in rabbits. However, neurotoxicity studies are required before these agents can be considered safe for clinical use. The aim of this study was to evaluate by histologic and blood–brain barrier (BBB) studies whether ketamine or midazolam could be used as an alternative to local anesthetics or opioids to produce spinal analgesia. Forty white New Zealand rabbits were randomly assigned to four groups of 10. In the conscious animal, 0.3 ml 0.9% saline solution, 1% lidocaine, 1% ketamine, or 0.1% midazolam was intrathecally injected intracisternally using a modification of the technique of Yaksh *et al.* Light and fluorescence microscopy were performed on transverse spinal cord sections by a neuropathologist unaware of the administered agents. All spinal cord section slides were scored within four zones: upper cervical, lower cervical, median thoracic, and lumbar segments. Spinal cord homogeneous lesions with higher scores than those of lidocaine-treated animals were considered abnormal. The BBB study showed evidence of neurotoxicity for ketamine, whereas light microscopy indicated no significant differences in comparison with saline and lidocaine. Midazolam-treated rabbits showed significant changes in both BBB and light microscopy studies. In view of these results, the intrathecal use of midazolam should be avoided in humans. Lesions observed following ketamine suggest the need for further experimental studies of the solvent and different ketamine enantiomers to establish definitively the safety of intrathecal free ketamine in humans. (Key words: Anesthetic techniques: spinal. Anesthetics, intravenous: ketamine. Anesthetics, local: lidocaine. Hypnotics, benzodiazepines: midazolam. Spinal cord: subarachnoid space. Toxicity: neurotoxicity.)

UNLIKE LOCAL ANESTHETICS, intrathecally or epidurally administered opioids produce selective spinal analgesia.[1] However, the use of opioids by these routes involves two major problems—respiratory depression and tolerance.[1] Advances in physiology and pain relief have suggested the existence of many pathways and transmitters. Development of spinal drugs[1] that optimize antinociceptive effects and minimize adverse effects therefore seem desirable as an alternative to opioids or local anesthetics. Investigators have demonstrated that agents such as ketamine or midazolam may be suitable alternatives to intrathecal opioids in morphine-tolerant cancer patients.[2,3]

Benzodiazepine-stereospecific binding sites are found in many human tissues, including the spinal cord,[4] and endogenous benzodiazepinelike substances have been discovered in human cerebrospinal fluid (CSF). Benzodiazepines may modulate the affinity of γ-aminobutyric acid (GABA) for its receptors while enhancing its control of chloride channel activity. Midazolam, the newly introduced benzodiazepine, is water-soluble. Thus, for the first time, topical application of midazolam to nervous tissue is feasible.[5] Intrathecal administration of midazolam has been shown to interrupt somatosympathetic reflexes in anesthetized dogs.[5] In dogs[5] and rats[6] this action does not seem to have a local anesthetic effect. A receptor mechanism is suggested because of the ability of a specific benzodiazepine antagonist to reverse the effect, but opiate receptors evidently are not involved[5,6] since the effects of midazolam are not antagonized by naloxone.[5,6] Midazolam has been successfully administered epidurally for pain in humans.[7] When intrathecally administered, it has abolished pain of somatic origin, produced selective sensory blockade, and blocked somatosympathetic reflexes.[2]

Ketamine hydrochloride, a potent analgesic and anesthetic agent,[8] appears to be an N-methyl-D-aspartate receptor agonist,[9] and its effects are partly antagonized by naloxone.[10] In humans, ketamine has been intrathecally administered for surgery of the lower limbs.[3] No motor block has been described for the agent when used alone. Ketamine has also been epidurally administered without side effects in patients suffering from intractable pain[11] or postoperative pain.[12] Otherwise, ketamine has shown local anesthetic properties in regional intravenous anesthesia.[13]

A study demonstrating a lack of neurotoxicity is necessary before these agents can be safely used in humans.[14] A previous study[15] evaluated the neurotoxicity of midazolam in rats but revealed many technical problems. Recently, histologic study after epidurally chronic administration of midazolam in the rabbit showed no changes.[16] Reports about ketamine neurotoxicity are sparse and inconsistent.[17–19] Many disturbances were noted after high concentrations of intrathecal ketamine in the rat.[17] Other

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Received from the Département d'Anesthésie-Réanimation Chirurgicale, Hôtel-Dieu, Laboratoire d'Anesthésie et †Laboratoire d'Anatomopathologie, Faculté de Médecine, Nantes, France. Accepted for publication March 4, 1991. Presented in part at the 1990 annual meeting of the American Society of Anesthesiologists, Las Vegas, Nevada.

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authors attributed the observed changes to difficulty in performing dural punctures.[18] The aim of the current study was to carry out histologic and blood–brain barrier (BBB) examinations in the rabbit to determine whether ketamine and midazolam produce spinal cord lesions. Results are compared to those using 0.9% saline solution (group control) and lidocaine, an agent with a good safety record.

Materials and Methods

This study was approved by our Institution Animal Investigation Committee. Forty white New-Zealand rabbits of either sex, weighing 2.7 ± 0.5 kg, were randomly assigned to four groups of 10: group S received 0.9% saline serum; group L received 1% lidocaine (Roger Bellon); group K received 1% ketamine (Parke Davis); and group M received 0.1% midazolam (Roche). Vehicles for the agents were distilled water for saline solution and lidocaine; chlorobutanol as the solvent for ketamine; and 10% hydrochloric acid with sodium hydroxide as the solvent for midazolam diluted with 0.9% saline solution just before use.

The animals were fasted the day before the study. Under local anesthesia, a femoral artery and vein were cannulated to provide arterial blood pressure monitoring, arterial blood gas samples, and a route for fluid administration. Five $ml \cdot kg^{-1}$ of 2% Evans Blue (Sigma, catalogue number E 2129) was intravenously injected 5 h before spinal injection. Percutaneous puncture of the intracisternal subarachnoid space through the atlantooccipital membrane was performed using a 22-G needle with the conscious animal in lateral position and the head flexed. The subarachnoid position of the needle was confirmed by aspiration of CSF. Then 0.3 ml 0.9% saline serum ($pH = 5$), 1% lidocaine ($pH = 6.7$), 1% ketamine ($pH = 4$), or 0.1% midazolam ($pH = 3.9$) was injected and the needle withdrawn immediately after injection.

In 20 of 40 animals (5 in each group), arterial blood pressure and an ECG for heart rate (HR) were continuously monitored on a multichannel recorder from cannulation of the femoral artery to the time of recovery from spinal injection. Arterial blood samples were withdrawn and arterial blood gases measured using an acid–base analyzer, and the respiratory rate (RR) was noted at four different times: after vascular cannula; after intravenous (iv) injection of Evans Blue; 3 min after spinal injection; and in the recovery period after spinal injection.

Animals were maintained in the erect position for 5 min. Ringer's lactate (Baxter) was infused intravenously when systolic blood pressure dropped to less than 90 mmHg. If respiratory depression occurred, the lungs were manually ventilated *via* a face mask. Arterial and venous catheters were withdrawn after recovery from spinal injection. The animals were killed by thiopental overdose on day 8. After complete bleeding, laminectomy was performed within 30 min after death. The dura was exposed, and 1 ml of a fixative containing 4% paraformaldehyde and 1.25% glutaraldehyde in buffer solution was subarachnoidally injected into the lumbar zone. The spinal cord with dura was carefully removed and immediately stored in the same fixative at 4° C. After paraffin embedding, spinal cords were sliced in transverse 6-μm sections. Microscopy examinations were performed on six slices in each segment (three in histologic examinations and three in BBB study) by a neuropathologist unaware of the intrathecal agent used. Spinal cords were scored for evidence of abnormalities or damage in four zones: upper cervical (C3–C4), lower cervical (C7–C8), median thoracic (T8), and lumbar (L4) segments.

Histologic Lesion Study

Light microscopy examination was performed on hematoxylin- and eosin-stained slides. A score of 0 indicated no abnormalities; 1, hemorrhage, glial cell reaction, and diffusion of the process over several areas in the same zone; and 2, extensive necrosis in the gray matter, hemorrhage, or great intensity of other lesions.

Blood–Brain Barrier Lesion Study

Evans Blue was revealed in unstained specimens by means of fluorescence microscopy. A score of 0 indicated no perivascular diffusion or spark around vessels; 1, slight diffusion around vessels; and 2, wide diffusion with loss of vessel outline.

The lesions were subsequently rated as normal or pathologic. Homogeneous lesions were those scored as the worst observed within a minimum of two different segments of spinal cord. An isolated lesion was not considered to be drug-mediated. Pathologic lesions were those scored higher than the worst in lidocaine-treated animals.

For the histologic and BBB studies, statistical comparisons were carried out using a contingency table and the chi-squared test and Fisher's exact test when appropriate. Hemodynamics and respiratory parameters were compared by analysis of variance (ANOVA) at different measurement times. $P < 0.05$ was considered significant.

During the week of observation after intrathecal injection, the rabbits were evaluated for any disturbances in locomotion while walking or any withdrawal responses to tail-flick and pin-prick stimulations. Patterns of urination and defecation as well as changes in aggressiveness or quietness and irregularities in food intake were noted.

Results

The scores of all spinal cord specimens are given in table 1. No sensory or motor blockade was noted with 0.9% saline serum. Lidocaine and ketamine induced spinal

TABLE 1. Histologic and Blood–Brain Barrier Scores in Four Zones

Animal Number	Weight (kg)	Agents	Histologic Lesion Score				Blood–Brain Barrier Lesion Score			
			UC	LC	MT	Lu	UC	LC	MT	Lu
1	2.20	S	0	0	0	0	0	0	0	0
3	2.35	S	0	0	0	0	0	0	0	0
7	2.55	S*	—	—	—	—	—	—	—	—
11	2.47	S	0	0	0	0	0	0	0	0
13	2.35	S	0	0	0	0	0	0	0	0
14	2.48	S	0	0	0	0	0	0	0	0
19	2.65	S	1	1	1	1	0	0	0	0
26	2.43	S	0	0	0	0	0	0	0	0
34	2.45	S	0	0	0	0	0	0	0	0
39	2.30	S	0	0	0	1	1	0	0	0
2	2.30	L	0	0	0	0	0	0	0	0
4	2.57	L	0	0	0	0	0	0	0	0
5	2.44	L	0	0	0	0	0	0	0	0
10	2.36	L*	—	—	—	—	—	—	—	—
16	2.88	L	1	1	1	1	1	1	1	1
22	2.85	L	0	1	2	1	0	2	0	1
24	2.84	L	1	1	0	0	0	1	0	1
29	2.64	L	0	0	0	0	0	0	0	0
33	2.90	L	0	0	0	0	0	0	0	0
40	2.16	L	0	0	0	0	0	0	0	0
8	2.35	K	1	1	1	1	2	2	2	0
9	2.82	K	2	1	1	0	2	2	2	0
15	2.44	K	0	0	0	0	0	0	0	0
17	2.53	K	2	2	1	0	0	0	0	1
18	2.76	K	1	1	0	0	1	1	1	1
20	2.59	K	0	0	0	0	1	0	2	1
27	2.79	K	0	0	0	1	0	0	0	0
28	2.86	K	0	0	0	0	0	0	0	1
35	2.72	K	0	0	0	0	0	0	0	0
36	2.74	K	1	2	2	1	2	2	1	2
6	2.82	M	2	2	2	2	2	2	2	1
12	2.72	M	0	0	0	0	1	1	1	1
21	3.27	M	0	0	0	1	2	1	0	1
23	3.21	M	2	2	2	2	1	1	1	1
25	3.21	M	0	0	0	0	1	1	1	1
30	3.17	M	1	1	1	1	2	2	2	2
31	3.00	M*	—	—	—	—	—	—	—	—
32	3.16	M	0	0	1	0	2	2	1	1
37	3.09	M	2	2	2	2	1	1	1	2
38	2.70	M	0	0	0	0	0	0	0	0

Segments: UC = upper cervical; LC = lower cervical; MT = median thoracic; Lu = lumbar.
Agents: S = saline solution; L = 1% lidocaine; K = 1% ketamine; and M = 0.1% midazolam.
* Results from three animals with cord injury (numbers 7, 10, and 31) were excluded.

anesthesia with respiratory depression in seven of ten animals receiving ketamine and in four of ten animals receiving lidocaine. In spinally anesthetized animals, lung ventilation, performed 3–5 min after spinal injection, lasted 10–20 min. Midazolam produced only a sensory block. Only animals receiving midazolam were quiet for a few hours after recovery from spinal injection. Ringer's lactate (10 ml · kg^{-1}) was infused when lidocaine or midazolam was injected in order to maintain arterial systolic blood pressure at nearly 90 mmHg. Between intrathecal injection and death, none of the animals presented obvious neurologic impairment or behavioral disturbances. During laminectomy three animals with cord injuries (blue pinpoint on dorsal roots induced by medullary puncture) but without obvious neurologic impairment were excluded from the study.

Lesions in the different studies were localized in the spinal cord within the deep gray and white matter. Fluorescence microscopic features were found mainly in capillaries and venules, with a few in small arteries.

Only one saline-solution-treated animal presented histologic homogeneous lesions of the spinal cord that scored as 1 without showing vascular disturbances. Two animals receiving lidocaine (animals 16 and 22) presented homogeneous histologic lesions and vascular changes. Animal 22 presented a median thoracic histologic lesion,

scored as 2, which was a large vacuolization in the area; this animal was considered normal.

When histologic or vascular changes were noted in the groups that received ketamine or midazolam, there was similar lesion intensity in the different zones.

In the group of animals receiving ketamine, animal 36 presented abnormal changes in both studies; animal 17 had only histologic lesions; and two others (animals 8 and 9) showed lesions only in the BBB study.

In midazolam-treated animals, two (animals 6 and 37) presented abnormal changes in both studies, one (animal 23) only in the histologic study, and three others (animals 21, 30, and 32) only in the BBB study.

The results of statistical analysis are summarized in table 2A (ketamine) and 2B (midazolam). In ketamine-treated rabbits, statistical differences between groups appeared in the BBB study, whereas the results of histologic study were considered normal. There were significant differences between groups for midazolam in both studies.

Results of hemodynamic changes are presented in table 3. No significant decrease in mean arterial blood pressure was observed after the different treatments. Heart rate and respiratory rate results are presented in tables 4 and 5. Measurements of arterial blood gas are summarized in table 6. In animals receiving lidocaine and ketamine, assisted ventilation was performed when a significant decrease of respiratory rate was observed, and hypoxemia was never observed.

Discussion

Direct spinal injection has not yet been performed in anesthetized animals because of the considerable danger of inadvertent damage to the spinal cord or spinal nerves by direct puncture in the subarachnoid space.[17,18] To avoid such difficulties a technique of chronic cannulation of the subarachnoid space has been developed in the rabbit[16,20] and the rat.[20,21] However, insertion of a catheter into the subarachnoid space could cause lacerations or abrasions of the spinal cord. Histopathologic light microscopy studies have shown extensive modifications due to the presence of an indwelling catheter in control animals infused only with saline solution.[22] Moreover, chronic spinal cannulation in various animal models has been shown to lead to changes, including fibrosis and lymphocytic infiltrations, around the catheter in the subarachnoid space.[22] In these circumstances, it is difficult to distinguish between drug-induced and catheter-induced modifications.

TABLE 2A. Number of Rabbits Receiving Ketamine in Histologic and Blood–Brain Barrier Studies

	Histologic Study		Blood–Brain Barrier Study	
Agent	Normal	Pathologic	Normal	Pathologic
S	9	0	9	0
L	9	0	9	0
K	8	2	7	3*

One rabbit in group S and one in L were excluded before examinations.
* $P < 0.04$ for ketamine *versus* saline solution or lidocaine.

TABLE 2B. Number of Rabbits Receiving Midazolam in Histologic and Blood–Brain Barrier Studies

	Histologic Study		Blood–Brain Barrier Study	
Agent	Normal	Pathologic	Normal	Pathologic
S	9	0	9	0
L	9	0	9	0
M	6	3*	6	3*

One rabbit in each group were excluded before examinations.
* $P < 0.05$ for midazolam *versus* saline solution or lidocaine.

We developed a rabbit model of spinal cord injury based on the technique (slightly modified) previously described by Yaksh and Rudy.[20] The rabbit, which is commonly used in neurotoxicity studies,[16,20,23] was chosen in preference to more costly animals such as the dog, sheep, or primate. Although it is impossible to perform blind percutaneous puncture in the rabbit lumbar region without causing trauma, this is easier in the larger cervical subarachnoid space. Moreover, an injection of 300 μl is easily tolerated by the rabbit[20] without risk of irreversible damage from increasing CSF pressure. Spinal injection in conscious animals also has the advantage of permitting observation of the onset of effects and the detection of inappropriate intravascular or intramuscular injections.

We chose clinically used concentrations for ketamine and midazolam. Bion has reported on the action of 1% ketamine in limb surgery,[3] and this concentration was chosen by Brock-Utne *et al.* in their experiments in the primate.[18,19] In the rat, use of intrathecal 5% ketamine led to histologic changes that were correlated with neurologic impairment.[17] In humans, ketamine did not produce neurologic changes in the one study in which it was evaluated.[3]

For midazolam, few patients have received a total intrathecal dose ranging from 0.5 to 2 mg in 3 ml of volume (0.06–0.16%).[2] The effects of 0.1% midazolam has been evaluated in a histologic study in the rat.[15]

The most commonly used fixative, which contains paraformaldehyde and glutaraldehyde, was essential to our technique. Evans Blue was chosen since histologic studies were performed by light microscopy, for which this dye is particularly suitable. Evans Blue is bound by normal serum proteins (mainly albumin) and provides a more accurate image of BBB lesions although it does not necessarily provide proof of the neurotoxicity of a drug.

TABLE 3. Hemodynamic Changes before and after Intrathecal Injection

Agent		T_1	T_2	T_3	T_4
0.9% Saline	SAP	92.5 ± 3.9	88.2 ± 4.6	88.5 ± 4.6	87.8 ± 8.5
	MAP	85.8 ± 9.2	84.9 ± 9.8	86.3 ± 20	82.6 ± 13.3
	DAP	79 ± 3.9	75.8 ± 5.8	75.5 ± 2.6	73 ± 5.6
Lidocaine	SAP	99 ± 5	98.5 ± 3.9	100.8 ± 17.2	93.7 ± 6.5
	MAP	89 ± 5	85.5 ± 5.5	87.5 ± 17.4	81.7 ± 7.8
	DAP	84 ± 5.9	79.1 ± 6.2	81.5 ± 17.7	75.7 ± 9.2
Ketamine	SAP	104.5 ± 16.4	105.8 ± 18.4	124 ± 13.7	97.6 ± 18.2
	MAP	91.7 ± 14.9	93.1 ± 15.9	113.3 ± 17.3	90.7 ± 8.8
	DAP	85.3 ± 14.4	86.7 ± 14.9	108 ± 19.2	87.3 ± 4.8
Midazolam	SAP	94.6 ± 11.2	101 ± 5.4	88.5 ± 13.2	90.6 ± 11
	MAP	79.1 ± 5.9	81.4 ± 5.6	72.7 ± 11.2	73.6 ± 7
	DAP	71.3 ± 5.8	71.5 ± 6.1	64.8 ± 10.5*	65.1 ± 5.9

Data are means ± SD.

Statistical comparisons were carried out by ANOVA. DAP after midazolam administration was significantly lower in T_3 compared with T_1 and T_2 (*$P < 0.02$). All the other comparisons were not significant.

T_1 = the time after vein and artery cannulations; T_2 = the time after iv injection of Evans Blue; T_3 = 3 min after subarachnoid injection of agents; T_4 = 30 min after injection, during the recovery period.

We differentiated BBB disturbances from classic histologic lesions, which were of various types and not always associated with the former. Slight hemorrhages around capillaries and venules were the main features of such lesions, and undefined conditions other than the introduction of foreign bodies into CSF may be implicated as causes.

The lesions observed were localized in the deep gray and white matter of the spinal cord, not only on the dorsal roots. The BBB disturbances were not localized in a terminal artery layer but involved mainly capillaries and venules; some involved small arteries. The dye was not detected in the pial network since it was probably diluted in the CSF.

Nerve lesions can be due to ischemic, traumatic, or toxic factors. We have evaluated the action of agents on vessels with vascular lesions by the fluorescence microscopy technique since staining could determine whether the neurotoxicity of a spinally injected agent was due to its vascular action. Increased endoneurial fluid pressure[24] due to spinal cord trauma can lead to possibly degenerative lesions. Rabbits found to have spinal cord injuries during laminectomy resulting from subarachnoid puncture were thus excluded from our study, even in the absence of clinical neurologic signs. Side effects were not evident in the hemodynamic or respiratory data that were recorded. The major hypothesis is that toxic effects were due to intrathecally administered agents, since lesions were observed mainly in the cervical segment and were distributed blindly.

After intrathecal saline serum injection, mild histologic homogeneous changes were found without vascular abnormalities in one rabbit. Two of nine animals in the group receiving lidocaine presented a few histologic lesions, the presence of which was associated with a slight diffusion of Evans Blue in the BBB study. Our data agree with those concerning the neurotoxicity of local anesthetic agents.[22,23] Slight abnormalities have also been reported in light microscopy studies of rabbit nerves exposed to saline solution and 2% lidocaine.[24] The isolated lesions observed in saline-solution-treated animals in one zone were not drug-mediated and were thus considered as normal (animal 39). The changes shown with histologic or BBB studies in animals that received lidocaine (animals 16, 22, and 24) were considered normal in view of the long safety record of this agent. The worst scores for the lesions were scores of 1. (Animal 22, with a score as 2, presented in only one segment associated lesions). Only scores above 1 were considered pathologic.

Our results after intrathecal ketamine are in agreement

TABLE 4. Heart Rate before and after Intrathecal Injection

Agent	T_1	T_2	T_3	T_4
0.9% saline	268 ± 16	268 ± 9	259 ± 3	248 ± 16
Lidocaine	260 ± 4	260 ± 7	266 ± 10	250 ± 15
Ketamine	247 ± 23	248 ± 26	249 ± 15	232 ± 17
Midazolam	267 ± 13	259 ± 13	239 ± 27	266 ± 13

Data are means ± SD. All comparisons are not significant.

TABLE 5. Respiratory Rate before and after Intrathecal Injection

Agent	T_1	T_2	T_3	T_4
0.9% Saline	68 ± 9.9	68.5 ± 9.7	59 ± 13.8	67 ± 5.9
Lidocaine	75.5 ± 9.3	75.5 ± 8.5	56.5 ± 6*	68 ± 8.5
Ketamine	69 ± 3.8	68.5 ± 4.1	33.5 ± 15.3*	66.2 ± 6.1
Midazolam	60 ± 9.7	61.5 ± 11	51.5 ± 6.2	66.2 ± 4

Data are means ± SD.

After ketamine intrathecal injection, respiratory rate was significantly dropped in T_3 *versus* all times (*$P < 0.05$). After lidocaine administration, respiratory rate was significantly decreased in T_3 *versus* T_2 (*$P < 0.05$).

TABLE 6. Arterial Blood Gas before and after Intrathecal Injection

Agent		T_1	T_2	T_3	T_4
0.9% Saline	Pa_{O_2}	108.8 ± 13.5	102.8 ± 18.8	110.3 ± 15.8	114 ± 24.8
	Pa_{CO_2}	33 ± 4.5	34.5 ± 4.5	31.5 ± 3	32.3 ± 2.3
	*p*H	7.29 ± 0.02	7.32 ± 0.04	7.31 ± 0.02	7.36 ± 0.06
Lidocaine	Pa_{O_2}	100.5 ± 12.8	135.8 ± 25.5	111.8 ± 12.8	127.5 ± 12
	Pa_{CO_2}	27.8 ± 3.8	18 ± 3.8*	22.5 ± 2.3	20.3 ± 3.8*
	*p*H	7.33 ± 0.01	7.32 ± 0.07	7.32 ± 0.05	7.38 ± 0.04
Ketamine	Pa_{O_2}	112.5 ± 26.3	91.5 ± 21	81 ± 25.5	109.5 ± 25.5
	Pa_{CO_2}	35.3 ± 5.3	36.8 ± 6.8	27.5 ± 6	33.8 ± 7.5
	*p*H	7.33 ± 0.05	7.31 ± 0.04	7.33 ± 0.04	7.32 ± 0.05
Midazolam	Pa_{O_2}	120 ± 42	126 ± 45.8	126 ± 53.3	126 ± 18.9
	Pa_{CO_2}	34.5 ± 3	30.8 ± 7.5	31.5 ± 1.5	30.8 ± 6
	*p*H	7.30 ± 0.04	7.24 ± 0.07	7.27 ± 0.05	7.27 ± 0.05

Data are means ± SD.
After intrathecal administration of lidocaine, Pa_{CO_2} was significantly decreased in T_2 and T_4 compared to T_1 (*$P < 0.01$).

with those of other authors.[18,19] Only two animals presented changes in both examinations. The other pathologic animals presented histologic or BBB changes. We observed respiratory depression. No hypoxemia or hypercapnia occurred because artificial ventilation was performed. The changes observed seem not to have been secondary to the systemic effects of the agent. However, previous animal studies of the potential ketamine neurotoxicity have proved somewhat contradictory.[17–19] Amiot *et al.*[17] found that one third of rats with limb paralysis after intrathecal ketamine injection had a focal degeneration with myelin loss but without traumatic cord injury. This may have been a toxic concentration effect on nervous tissue because they used a concentration of 5% ketamine compared to the 1% maximum in our study and that of Brock-Utne *et al.*[18,19] Brock-Utne *et al.* carried out their study in primates and found that intrathecal ketamine with or without preservative induced edema in a few nerve roots in all animals. They also determined that two of eight animals receiving ketamine had focal degeneration with loss of myelin and axoplasm. Since they noted that injection conditions were difficult, these lesions could have been due to spinal laceration during lumbar puncture. Moreover, these authors did not attribute any histologic changes to the use of preservative.[18] Only light microscopy was performed in each of these studies.[16–18] Our findings for ketamine do not indicate specific histologic changes when compared to those for use of saline solution or lidocaine, whereas our vascular study showed statistical differences as compared to results with saline solution or lidocaine ($P < 0.04$), thus suggesting a specific BBB disturbance. The ketamine preservative we used was chlorobutanol, whereas Brock-Utne *et al.*[18,19] used benzethonium chloride. Since these toxic effects may have been due to the drug itself or to the antioxidant, further studies with different ketamine components are required to determine whether free ketamine induces the same vascular disturbances.

After intrathecal midazolam, histologic and BBB studies showed significant changes in two of nine animals (animals 6 and 37), whereas two other animals presented lesions only in the histologic (animal 23) or the BBB study (animal 21). Systemic effects occurred after use of intrathecal midazolam in rabbits as sedation and hypotension. It must be hypothesized that the observed microscopic changes were due to topical toxicity or systemic side effects of the agent. In our study, rabbit lesions seem not to have been due to spinal ischemia since the animals did not have severe hypotension. Neurotoxicity could be presumed in two animals (animals 6 and 37), but there was no evidence of it in the other rabbits receiving midazolam. Another hypothesis that could explain the observed changes in the BBB study is the ability of hypotension to disrupt the BBB. To our knowledge no data have been reported regarding this possibility, and we did not induce deliberate hypotension during the experiment. However, this point merits further investigation. Determination of midazolam neurotoxicity, first studied by Auroy *et al.*,[15] proved difficult because of technical problems, and the existence of catheter-induced lesions did not permit definitive conclusions. Their study, based only on histologic examinations, is in agreement with our results in light microscopy. Recently, in chronic epidurally midazolam-treated animals,[16] histologic examinations showed no specific changes. Perhaps 10% hydrochloric acid as the midazolam vehicle may be incriminated as a factor in producing neurotoxicity.

The acidity of spinally administered solutions is apparently not sufficient to produce specific histologic lesions.[25]

It would appear that further experimental studies of

both ketamine enantiomers as well as the solvent are necessary to determine whether intrathecal free ketamine is a safe means of producing analgesia in humans. The use of intrathecal midazolam in humans should be avoided because the histologic and vascular lesions caused by this agent suggest a neurotoxic effect.

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