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Is Ketamine or Its Preservative Responsible for Neurotoxicity in the Rabbit?

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Background: **Although ketamine has been administered spinally in humans, previous neurotoxicity studies have shown that it can induce spinal cord lesions in various animal models. The aim of this work was to evaluate by histologic and blood-brain barrier studies whether different components of the commercial ketamine solution might be responsible for the microscopic lesions observed.**

Methods: **Forty white New Zealand rabbits were randomly assigned to four groups of 10. One-percent preservative-free ketamine (0.3 ml), 1% d-ketamine, 0.05% chlorobutanol, and 1% lidocaine were intrathecally injected through the atlantooccipital membrane. Laminectomy was performed on day 8, and the dura was preserved using paraformaldehyde-glutaraldehyde fixative. Light and fluorescence microscopy were performed on transverse spinal cord sections by a neuropathologist unaware of injected agents used. Specimens were then graded as normal or abnormal as compared with a control group receiving lidocaine.**

Results: **Isomers of ketamine did not induce spinal cord lesions in either study, but chlorobutanol (the preservative used in the ketamine solution) induced significant severe spinal cord lesions in both studies.**

Conclusions: **The appearance of spinal cord lesions after intrathecal chlorobutanol strongly suggests that this preservative is responsible for apparent toxicity of ketamine and therefore should not be used in any solution intrathecally injected into humans. (Key words: Anesthetic techniques: spinal. Anesthetics, intravenous: ketamine. Anesthetics, local: lidocaine. Preservatives: antioxidant; chlorobutanol. Spinal cord: subarachnoid space. Toxicity: neurotoxicity.)**

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KETAMINE has been administered epidurally for pain relief in humans[1,2] with no side effects such as respiratory depression, urinary retention, or pruritus as are seen following epidural opioids. The exact mechanism of spinal analgesia is unknown, but the sedative effect after a large dose of epidural ketamine is apparently due to vascular uptake and systemic action.[1,2] Administered intrathecally in humans, 50 mg ketamine produced surgical anesthesia associated with sensory blockade lasting 1 h, as well as motor blockade when associated with epinephrine.[3] Although no clinical neurologic lesions were noted after spinal administration of ketamine in these reports,[1–3] intrathecal ketamine cannot be considered for routine clinical use because of the suggestion from animal studies of neurotoxicity.[4–7]

In early reports using light microscopy, Brocke-Utne *et al.* found edema in a few nerve roots in all ketamine-treated primates.[4,5] Two animals presented focal degeneration, with loss of myelin and axoplasm, attributed to spinal laceration during lumbar puncture.[4] Auroy *et al.* found that rats, after intrathecal injection of 5% ketamine, had limb paralysis without traumatic cord injury and showed focal degeneration with myelin loss.[6] This may have been a toxic concentration effect on nervous tissue.[6]

In recent investigations in the rabbit,[7] classic histologic studies were supplemented with the blood-brain barrier (BBB) studies using fluorescence microscopy. Evidence of ketamine neurotoxicity was found in the BBB studies, whereas light microscopy indicated no significant differences compared to results with saline solution and lidocaine.[7] It was suggested that ketamine (optical isomers, free ketamine) or its preservative, chlorobutanol, was the most likely cause of toxicity.[7] In previous studies, no histologic changes were attributed to another preservative, benzothenium chloride.[4,5]

The purpose of the present study was to carry out histologic and BBB examinations in rabbit, using a previously described neurotoxicity model,[7] to determine

whether preservative-free ketamine, d-enantiomer, or its solvent, produce spinal cord lesions.

Materials and Methods

The study was approved by our Institution's Animal Investigation Committee. Forty white New Zealand rabbits weighing 2.8 ± 0.3 kg, were randomly assigned to four groups of 10: group pf-K received 1% preservative-free ketamine (pf-K; Parke Davis, Courbevoie, France), group d-K received 1% d-enantiomer of ketamine (d-K; Parke Davis), group C received 0.05% chlorobutanol (C; Parke Davis), and group control L received 1% lidocaine (L; Roger Bellon, Nevilly sur Seine, France). Distilled water was the vehicle for each administered agent.

Animals 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. In the conscious animal, percutaneous puncture of the intracisternal subarachnoid space through the atlantooccipital membrane was performed using a 22-G needle, as previously described.[7] The subarachnoid position of the needle was confirmed by aspiration of cerebrospinal fluid before injection of 0.3 ml 1% lidocaine (pH = 6.7), 1% preservative-free ketamine (pH = 4), 1% d-ketamine (pH = 4), or 0.05% chlorobutanol (pH = 3.9). The needle was withdrawn immediately after injection. Animals were maintained in an erect position for 5 min.

In all animals, arterial blood pressure and heart rate were continuously monitored on a multichannel recorder from the time of the femoral artery cannulation until recovery from spinal injection. Arterial blood samples were withdrawn for blood gas analysis, and respiratory rate were noted at the following times: after vascular cannulation, 3 min after spinal injection, and in the recovery period after spinal injection.

Lactated Ringer's solution (Baxter) was infused intravenously when systolic blood pressure decreased 25% from baseline value. When respiratory depression occurred, the lungs were manually ventilated *via* a face mask. Arterial and venous catheters were removed after recovery of spinal injection.

On day 8, 2% Evans Blue® (Sygma, E 2129) 5 ml/kg was intravenously injected 2 h before killing of the rabbit with an overdose of thiopental. Animals with spinal cord injuries (presenting blue pinpoints on dorsal cervical roots) were excluded during laminectomy, which 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 a phosphate buffer solution (pH = 7.2) was subarachnoidally injected into the lumbar zone. 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, 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 (C3–4), lower cervical (C7–8), median thoracic (T8), and lumbar (L4) segments.

From the time of spinal injection to death (day 8), rabbits were evaluated by an assistant unaware of intrathecally administered agent for locomotion disturbances while walking or responses to tailflick and pinprick stimulations. Animal behavior patterns including change in aggressiveness or quietness, food intake irregularities, urination, and defecation were also noted.

Histologic Lesion Study

Hematoxylin- and eosin-stained slides were examined in light microscopy. 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, large hemorrhage, or other lesions such as axonal degeneration.

Blood-brain Barrier Lesion Study

Evans Blue was revealed on unstained specimens with 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.

Vasodilation was considered when capillaries and venulae were abnormally apparent.

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

For the histologic and BBB studies, statistical comparisons were carried out using a contingency table and, when appropriate, chi-square test and Fisher's exact tests. Hemodynamic and respiratory parameters were compared by one-way ANOVA with repeated

measurements ($P < .05$ being considered as significant).

Results

No sensory or motor blockade was noted after intrathecal chlorobutanol administration. Lidocaine, preservative-free ketamine, or d-ketamine induced spinal anesthesia with respiratory depression in, respectively, two, three, and four animals (group L, animals 7 and 9; group pf-K, animals 1, 4, and 6; group d-K, animals 2, 3, 7, and 9). In these animals, lung ventilation was performed 3–5 min after spinal injection, for 10–20 min. None of the animals presented obvious neurologic impairment or behavioral disturbances between intrathecal injection and death. Five animals with spinal cord injuries were excluded from the study.

Astrogliosis (fig. 1) was present in all segments of spinal cord, independent of BBB lesions, vasodilation, or hemorrhage. It was related to weight and could be considered a reflection of age and independent of experiment conditions.

The scores of all spinal cord histology are given in table 1. When lesions occurred, they were not localized in any particular area in the spinal cord. Histologic lesions were mainly localized in posterior dorsal roots of spinal cord in white matter structures as BBB rupture and axonal degeneration (fig. 2), and gray structures in zona posterior of dorsal horn (slices III–V). Fluorescence microscopic features were found mainly in capillaries, venulae, and small arteries. Venous and arterial vessel outlines appeared to be optically normal.

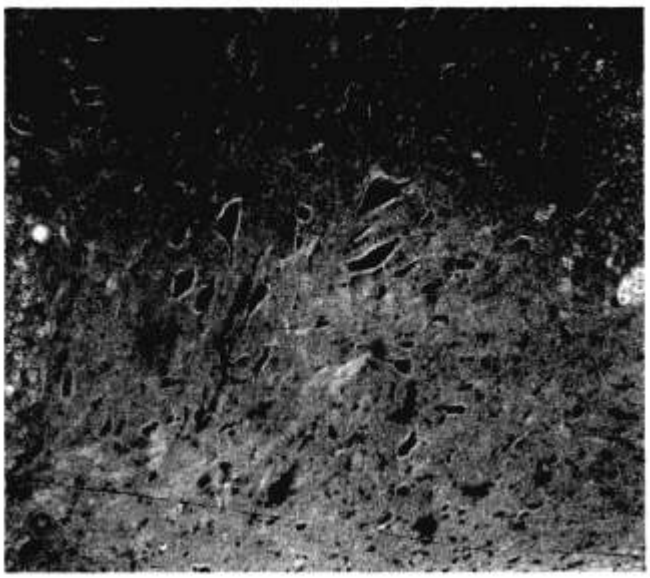

Fig. 1. Hematoxylin- and eosin-stained slide (40× magnification). Dorsal horn of upper cervical segment in chlorobutanol-treated rabbit (animal no. C_2). Vasodilation and blood suffusion, astrogliosis nonspecific related to age.

Cord slices showed no changes after intrathecal lidocaine in both BBB and histologic studies, and all animals were considered normal. Only one animal in each group receiving pf-K or d-K had homogeneous lesions in BBB study, as revealed by slight diffusion in the white matter in upper segments.

Three animals receiving chlorobutanol were considered normal in both studies, and one rabbit presented no pathologic lesions in histologic study. The spinal cord of all other animals were considered to have pathologic changes. Some of them showed mild vascular lesions (fig. 3), but the main lesions observed in the BBB study were loss of vessel outline in dorsal and anterior segments (fig. 4). These vascular changes were associated with the worst lesions in the histologic study.

The results of statistical analysis for BBB and histologic studies are summarized in table 2. Only group C induced statistically significant lesions when compared with groups L, pf-K, and d-K.

There was no significant decrease in mean arterial blood pressure, heart rate, or respiratory rate after the different treatments (tables 3 and 4). Arterial blood gas measurements are shown in table 5. No hypoxemia occurred. In chlorobutanol-treated animals, severe lesions were not associated with significant changes in hemodynamics parameters or arterial blood gas measurements.

Discussion

The results of our study showed significant changes in histologic and BBB studies following chlorobutanol, which were not associated with isomers of ketamine without preservative.

When 5% intrathecal ketamine was used in rats without traumatic spinal cord injury, histologic changes attributed to the toxic effect of the agent were induced.[6] The ketamine concentrations tested in previous experimental neurotoxicity studies were similar to those clinically used in humans.[4,5,7] Brock-Utne *et al.* found no histologic differences when ketamine was used with or without a preservative, 0.01% benzethonium chloride.[3] In a previous study involving rabbits, we noted statistically significant differences for ketamine with preservative as compared to control groups receiving

Table 1. Histologic and Blood–Brain Barrier Scores in Four Zones: Upper Cervical, Lower Cervical, Median Thoracic, and Lumbar Segments

			Histologic Lesion Score				Blood–Brain Barrier Lesion Score			
No.	kg	Agents	Upper Cervical	Lower Cervical	Median Thoracic	Lumbar	Upper Cervical	Lower Cervical	Median Thoracic	Lumbar
L_1	2.52	L	0	0	0	0	0	0	0	0
L_2	2.51	L*	—	—	—	—	—	—	—	—
L_3	2.26	L	0	0	0	0	0	0	0	0
L_4	2.36	L	0	0	0	0	0	0	0	0
L_5	2.53	L	0	0	0	0	0	0	0	1
L_6	2.24	L	0	0	0	0	0	0	0	1
L_7	2.67	L	0	1	0	0	0	0	0	0
L_8	2.43	L	0	0	0	0	0	0	0	0
L_9	2.51	L	0	0	0	0	0	0	0	0
L_{10}	2.72	L	0	0	0	0	0	0	0	0
pf-K_1	3.10	pfK	0	1	1	0	0	2	2	1
pf-K_2	2.64	pfK	0	1	0	0	0	0	0	0
pf-K_3	2.70	pfK	1	0	0	0	0	0	0	0
pf-K_4	2.31	pfK	0	0	0	0	0	0	0	0
pf-K_5	2.25	pfK	0	0	0	0	0	0	0	0
pf-K_6	2.48	pfK	0	0	0	0	0	0	0	0
pf-K_7	2.53	pfK	0	1	0	0	1	0	0	0
pf-K_8	2.45	pfK	0	0	0	0	0	0	0	0
pf-K_9	2.51	pfK	0	0	0	0	0	0	0	0
pf-K_{10}	3.10	pfK*	—	—	—	—	—	—	—	—
d-K_1	2.33	dK	0	0	0	0	0	0	0	0
d-K_2	2.36	dK	0	0	0	0	0	0	0	0
d-K_3	2.56	dK*	—	—	—	—	—	—	—	—
d-K_4	2.92	dK	0	1	0	0	1	0	0	0
d-K_5	3.32	dK*	—	—	—	—	—	—	—	—
d-K_6	2.13	dK*	—	—	—	—	—	—	—	—
d-K_7	2.65	dK	0	1	0	1	1	2	2	1
d-K_8	2.79	dK	0	0	0	0	0	0	0	0
d-K_9	2.40	dK	0	0	0	0	0	0	0	0
d-K_{10}	3.15	dK	0	1	0	0	0	0	0	0
C_1	2.64	C	0	1	1	0	0	0	1	0
C_2	2.26	C	2	2	1	1	2	2	2	2
C_3	2.34	C	2	2	2	2	2	2	2	2
C_4	2.22	C	1	1	1	1	1	1	2	1
C_5	2.45	C	0	0	0	0	1	0	1	0
C_6	2.65	C	0	1	0	1	1	0	1	1
C_7	3.10	C	2	2	2	2	2	2	2	2
C_8	2.26	C	2	2	1	1	2	2	2	2
C_9	2.38	C	2	2	2	1	1	2	2	1
C_{10}	2.49	C	1	2	2	1	2	2	2	0

Results from five animals with cord injury (No. L_4, pf-K_{10}, d-$K_{3,5,6}$) were excluded.

* Animals excluded.

lidocaine or saline solution.[7] These animals presented BBB disturbances not associated with histologic changes.[7] In the present study, concentrations were equivalent to those routinely used in commercial ketamine solutions and similar to those of previous experimental studies: 1% for d-ketamine or preservative-free ketamine and 0.05% for chlorobutanol.

Direct spinal injection, as previously described,[7] allowed observation of clinical spinal anesthesia. This technique avoids the nonspecific lesions observed after chronic cannulation of the subarachnoid space, and we did not observe inflammatory reactions in histologic study in the present study. As nerve lesions can be due to traumatic factors, rabbits with spinal cord injuries resulting from spinal puncture, observed during laminectomy, were excluded.

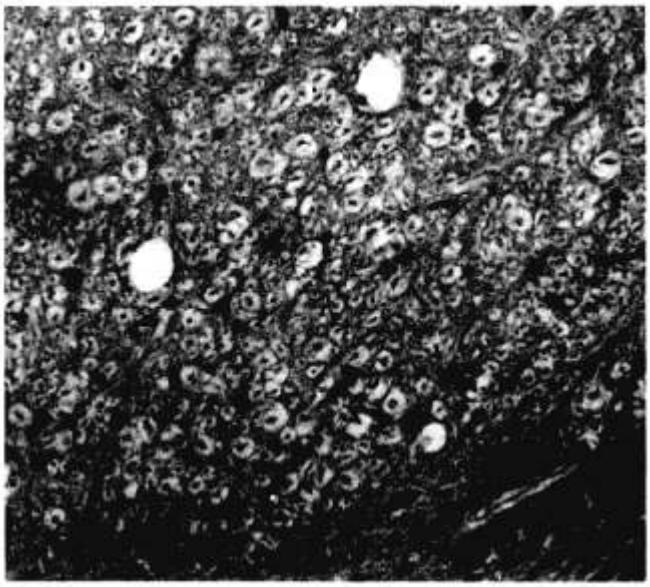

Fig. 2. Hematoxylin- and eosin-stained slide (100× magnification). Ventral root of upper cervical segment of chlorobutanol-treated rabbit (animal no. C_3). Axonal degeneration.

Our results with lidocaine were similar to those in our previous experimental study.[7] A slight modification in dye injection (2 h before death compared with 8 days before in the former study[7]) had no effect on the results obtained.

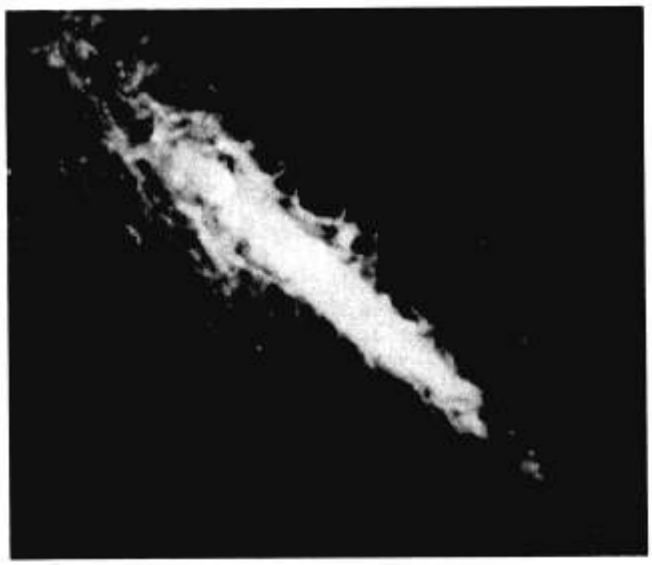

Fig. 3. Fluorescent microscopy (350× magnification). Lateral root of upper cervical segment of chlorobutanol-treated rabbit (animal no. C_7). Slight diffusion of dye (Evans Blue) around vessel.

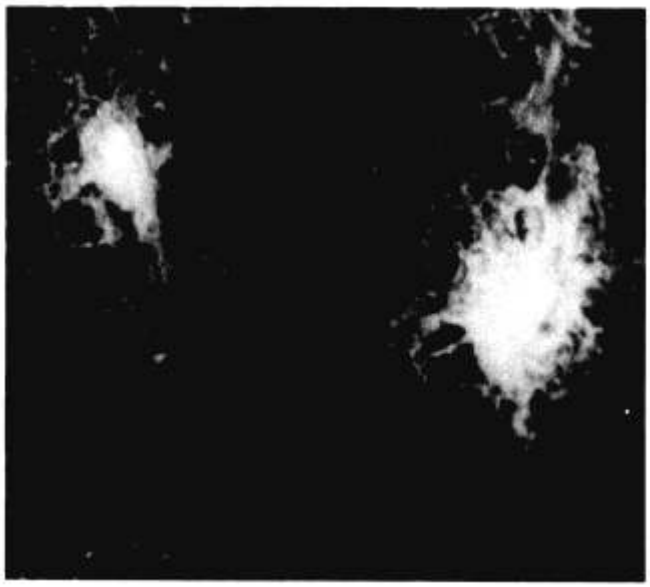

Fig. 4. Fluorescent microscopy (350× magnification). Dorsal root of upper cervical segment of chlorobutanol treated rabbit (animal no. C_3). Large diffusion of dye (Evans Blue) with loss of vessel outlines.

We suggested in our previous study that ketamine with a preservative might have neurotoxic effects on nerve tissue in the rabbit.[7] In the present study, the main lesion sites were in cervical segments. It would seem that the lesion detected not only in the peripheral area of the spinal cord but also in deeper structures in both histologic and BBB studies was due to a neurotoxic effect of agent rather than to systemic side effects. When lesions appeared in lumbar segments, they were also present in rostral segment of the spinal cord. Histologic lesions were found in different areas of the ventral or dorsal roots of spinal cord, whereas BBB disturbances were not localized in a terminal artery layer but in-

Table 2. Statistics

	Histologic Study		Blood–Brain Barrier Study	
Group	Normal	Pathologic	Normal	Pathologic
L	9	0*	9	0‡
pf-K	9	0*	8	1†
d-K	7	0†	6	1†
C	4	6	3	7

Values are the number of rabbits receiving preservative-free ketamine, *d*-ketamine, or chlorobutanol in histologic and blood–brain barrier studies.

Comparisons *versus* chlorobutanol: * $P < .02$; † $P < .04$; ‡ $P < .01$. Other comparisons were NS.

Table 3. Mean Arterial Blood Pressure (MAP) and Heart Rate (HR) Changes Before and After Intrathecal Injection

Agent	T_1	T_2	T_3
Lidocaine			
MAP (mmHg)	89 ± 5	88 ± 17	82 ± 8
HR (beats/min)	288 ± 12	265 ± 8	260 ± 14
Preservative-free ketamine			
MAP (mmHg)	92 ± 15	113 ± 17	91 ± 9
HR (beats/min)	274 ± 13	261 ± 12	260 ± 14
d-Ketamine			
MAP (mmHg)	91 ± 9	100 ± 11	94 ± 9
HR (beats/min)	280 ± 10	258 ± 15	253 ± 14
Chlorobutanol			
MAP (mmHg)	86 ± 8	87 ± 8	89 ± 8
HR (beats/min)	275 ± 18	268 ± 16	273 ± 9

Results are expressed as mean ± SD.

T_1 = time after vein and artery cannulations; T_2 = 3 min after subarachnoid injection of agents; T_3 = 30 min after injection, during the recovery period. No statistically significant differences were noted in all comparisons.

volved mainly capillaries, venulae, and small arteries. Erythrodiapedesis resulting in suffusion without parietal lesions was not considered a hemorrhage and could be influenced by death conditions.

In our experiments, rabbits never had clinical neurologic impairment after intrathecal injection of ketamine isomers, contrary to the first reports on the use of ketamine with no preservative.[4] However, in that report the pathology was revealed by histologic studies.[4] Our results in animals receiving preservative-free ketamine or d-ketamine were similar to those in lidocaine-treated animals. Histologic lesions were never observed, but one animal in each isomer group presented slight modifications in BBB study. The lesions observed in our previous study after ketamine administration were not found in the present experiment and statistical comparisons in the BBB study revealed no significant effects for the isomers.

Table 4. Respiratory Rate Before and After Intrathecal Injection

Agent	T_1	T_2	T_3
Lidocaine	70 ± 8	66 ± 8	62 ± 8
Preservative-free ketamine	72 ± 6	57 ± 19	62 ± 13
d-Ketamine	69 ± 7	61 ± 24	59 ± 15
Chlorobutanol	68 ± 7	67 ± 9	71 ± 6

Results are expressed as mean ± SD.

T_1 = time after vein and artery cannulations; T_2 = 3 min after subarachnoid injection of agents; T_3 = 30 min after injection, during the recovery period. No statistically significant differences were noted in all comparisons.

Table 5. Arterial Blood Gas Before and After Intrathecal Injection

Agent	T_1	T_2	T_3
Lidocaine			
Pa_{O_2} (mmHg)	105 ± 16	110 ± 10	115 ± 15
Pa_{CO_2} (mmHg)	28 ± 4	27 ± 4	24 ± 3
*p*H	7.33 ± 0.02	7.32 ± 0.05	7.35 ± 0.05
Preservative-free ketamine			
Pa_{O_2} (mmHg)	100 ± 13	91 ± 18	109 ± 18
Pa_{CO_2} (mmHg)	31 ± 8	27 ± 8	27 ± 5
*p*H	7.33 ± 0.02	7.33 ± 0.02	7.33 ± 0.04
d-Ketamine			
Pa_{O_2} (mmHg)	102 ± 14	98 ± 20	112 ± 20
Pa_{CO_2} (mmHg)	29 ± 8	25 ± 7	29 ± 7
*p*H	7.33 ± 0.01	7.33 ± 0.03	7.34 ± 0.07
Chlorobutanol			
Pa_{O_2} (mmHg)	100 ± 12	102 ± 13	102 ± 8
Pa_{CO_2} (mmHg)	28 ± 5	25 ± 6	26 ± 4
*p*H	7.32 ± 0.07	7.33 ± 0.06	7.33 ± 0.02

Results are expressed as mean ± SD.

T_1 = time after vein and artery cannulations; T_2 = 3 min after subarachnoid injection of agents; T_3 = 30 min after injection, during the recovery period. No statistically significant differences were noted in all comparisons.

Intrathecal injection of preservative, chlorobutanol, led to extensive lesions in both histologic and BBB studies. Histologically, hemorrhage and extensive necrosis in gray structures were apparent in 60% of the spinal cord slides. The lesions revealed in the BBB study were loss of vessel outline in the spinal cord of the same animals in which histologic lesions were observed. Both histologic and vascular changes were similar in the same animals. Because no hypoxic or hemodynamic changes occurred, it would seem that these lesions in the same rabbits were due to a neurotoxic or vasotoxic effect of chlorobutanol. Scores obtained after ketamine with preservative[7] in our earlier study were lower than those found for the preservative alone in the present study.

Chlorobutanol is added to ketamine solution as an antibacterial and antifungal agent and is also a mild sedative and local analgesic agent when used at a concentration of 0.5%.[8] However, in our study these properties were not strong enough to induce an anesthetic spinal effect when used at 0.05%, whereas preservative-free ketamine or d-ketamine injected alone showed spinal anesthetic properties similar to those of commercial ketamine solution tested in previous studies.

In the present study, we observed lesions with pre-

servative chlorobutanol (pH = 3.9) but not with active components. Only 1% preservative-free ketamine and 1% d-isomer were tested in the present study, because this concentration was chosen in previous report. As Amiot *et al.*[6] reported with the toxic effect in nervous tissue of 5% ketamine (containing different isomers and chlorobutanol), further experimental studies with high concentrations such as 5% are needed to determine whether ketamine isomers produce no spinal lesions and provided anesthetic effects before its spinal administration in humans.

Our investigations strongly suggest that chlorobutanol is responsible for the apparent spinal cord toxicity associated with ketamine. If these results in animal experiments can be extrapolated to humans, chlorobutanol should not be included in an agent that may enter the subarachnoid space. In our animal model, 1% preservative-free ketamine or 1% d-ketamine induced spinal anesthesia as effectively as ketamine with a preservative but did not produce pathologic spinal cord disturbances apparent in microscopic examinations in the rabbit.

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