Original Contribution Kitasato Med J 2015; 45: 45-52

Comparison of neurotoxicity of intrathecally administered fentanyl and bupivacaine in rats

Tamie Takenami,[1] Sayano Fukushima,[1] Yoshihiro Nara,[1] Saburo Yagishita,[2] Sumio Hoka,[3] Yuki Nagahara,[1] Kazutaka Tanaka,[1] Hirotsugu Okamoto[1]

[1] Department of Anesthesiology, Kitasato University School of Medicine
[2] Department of Pathology, Kanagawa Rehabilitation Center, Kanagawa
[3] Department of Anesthesiology, Kyushu University School of Medicine, Fukuoka

Background: Fentanyl is intrathecally used as an adjuvant to bupivacaine in spinal anesthesia, as well as labor analgesia, and in day surgery. The aim of this study was to determine the separate neurotoxic effect of each drug using histological analysis.
Methods: Rats (N = 39) received fentanyl at 0.12 μl/g body weight (0.05, 0.5, and 1 mg/ml) or bupivacaine (5, 25, and 50 mg/ml) dissolved in saline via an intrathecal catheter. Saline was used as the control solution. Walking behavior and sensory threshold were used as neurofunctional tests. Seven days after the intrathecal injection, the L2 spinal cords, of each rat, with both anterior and posterior roots, including the dorsal ganglion and cauda equina, were examined histologically.
Results: No histological abnormalities were observed in any of the rats treated with fentanyl (0.05, 0.5, or 1 mg/ml) or bupivacaine (5 or 25 mg/ml). However, axonal degeneration originating from the posterior root, extending to the posterior white matter, was observed in rats treated with 50-mg/ml bupivacaine. Significantly higher sensory thresholds were observed in rats with 1 mg/ml fentanyl, or 50-mg bupivacaine at 2 hours after the injection. The higher thresholds gradually disappeared in rats with fentanyl after 1 hour even at 1 mg/ml, but remained in rats with 50-mg/ml bupivacaine after 2 hours, and significantly decreased at 7 days after the injection. The rats could walk normally within 15 minutes, 1 hour before, and 1 hour after the injection of fentanyl (0.05, 0.5, and 1 mg/ml), respectively, and within 1, 2, and 4 hours of bupivacaine (5, 25, and 50 mg/ml), respectively. Transient apnea was only observed in 1 rat treated with 0.05-mg/ml fentanyl, while both transient apnea and muscle rigidity were observed in rats treated with 0.5- and 1-mg/ml fentanyl. No rats treated with bupivacaine (5, 25, or 50 mg/ml) showed both side effects.
Conclusions: Our results indicated that intrathecal fentanyl does not cause any neurotoxic changes even at more than 40 times the clinical concentration (1 mg/ml), whereas bupivacaine causes nerve damage even when applied at 10 times the clinical concentration (50 mg/ml) in the spinal rat model. Side effects such as respiratory depression and muscle rigidity were seen in rats in the fentanyl group, even at 0.05 mg/ml. These results suggested that intrathecal fentanyl has strong side effects but low neurotoxicity because of the absence of morphological neurotoxicity even at high concentrations, whereas intrathecal bupivacaine induced sensory disturbance associated with axonal degeneration.

Key words: histological analysis, posterior root, axonal degeneration, posterior column, neurotoxicity

Introduction

The analgesic effects of spinal anesthesia are enhanced by intrathecal administration of fentanyl in addition to local anesthetics.[1,2] Moreover, a mixed solution of fentanyl and local anesthetics produces satisfactory analgesia with a smaller dose of local anesthetics, compared with a local anesthetics solution alone. Reduction in the dose of local anesthetics is expected to help shorten motor block duration,[3] and prevent hypotension or excessively high infusion volume by attenuating sympathetic nerve block.[4] Therefore, a mixed solution of fentanyl and local anesthetics is not only advantageous for obstetric anesthesia,[5,6] lower limb

Received 4 October 2014, accepted 22 December 2014
Correspondence to: Tamie Takenami, Department of Anesthesiology, Kitasato University School of Medicine
1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan
E-mail: takenami@med.kitasato-u.ac.jp

surgery,[7] and day surgery[5,8] due to reduction of motor block, but can also be used to maintain stable hemodynamics during surgery on elderly patients by reducing sympathetic nerve block.[4] Thus, the addition of fentanyl to local anesthetics has been frequently used in spinal and epidural anesthesia.

Attention has focused recently on the potential neurotoxic effects of certain local anesthetics. These neurotoxic effects can sometimes induce transient neurologic symptoms (TNS), which induce dysesthesia, such as pain, sores, or cramping in buttocks and lower limbs after spinal anesthesia. The reported incidence of TNS with lidocaine and bupivacaine are 30% and 0－3%, respectively.[8-10] To our knowledge, the incidence of TNS after using a mixed solution of fentanyl and local anesthetics has not been reported. It is noteworthy that in any neurologic disorder, including TNS that develops after the administration of a mixed solution, determination of the culprit drug responsible for the neurologic symptoms is often difficult. The purpose of the present study was to compare the neurotoxicity of fentanyl and bupivacaine and to estimate the contribution of each drug to neurologic impairment.

Materials and Methods

The Ethics Committee on Animal Research of Kitasato University School of Medicine approved the study protocol. Experiments were conducted in 39 male Wistar rats (8－10 weeks old, body weight 246－302 g). The animals were housed 2 per cage in the experimental facility for 1 week before the experiment and were maintained under a 12:12 hour light-dark cycle at a room temperature of 22℃. They were allowed free access to food and water.

Surgical procedure for intrathecal catheterization

Rats were anesthetized with isoflurane (2－3%), and the subarachnoid space was cannulated with a polyethylene tube (0.6 × 700 mm) through the atlantooccipital membrane using the modified method of Yaksh and Rudy.[11] The tip of the catheter was advanced 7.5 cm caudally to the L1 level to examine the L2 spinal cord without catheter injury, while the other end was fixed in the subcutaneous tissue to avoid dislodgement of the catheter. The rats were allowed to recover and the test drug was injected 1 week later. Rats showing symptoms of traumatic nerve damage due to catheter insertion were excluded from further experiments.

Intrathecal administration of anesthetic agents

At day 7 after intrathecal catheterization, the rats were divided into 7 groups based on the injected drug. Rats received 0.05, 0.5, or 1 mg/ml fentanyl (Daiichi Sankyo Propharma, Tokyo) dissolved in saline solution, or 5, 25, or 50 mg/ml bupivacaine (Astra Zeneka, Tokyo) dissolved in saline, or saline solution alone as a control. Each solution was prepared aseptically in the morning of the injection day. The total volume injected was 0.12 μl/g body weight in addition to 6 μl for the dead space of the catheter as in previous methods.[12-15] Under inhalational isoflurane anesthesia delivered through a face-snout mask, we opened the skin to expose the subcutaneously embedded intrathecal catheter, and injected each solution manually through the catheter over a 15-second period. Just before the drug injection, isoflurane inhalation was stopped (total inhalation time was <5 minutes), and the wound was closed leaving the catheter under the skin. The rats were allowed to breathe the air in the room during recovery from the anesthesia. The side effects induced by the intrathecal fentanyl or bupivacaine were also recorded.

Recovery time to ambulation

The behavior of each rat was evaluated by analyzing its ability to walk with or without limitation. Evaluation was performed at 0.25, 0.5, 1, 2, 3, and 4 hours after intrathecal injection on the day of injection (post-injection day [PID] 0) and every subsequent morning from PID 1 to PID 7. The recovery time to normal ambulation was scored as follows: 0 = normal ambulation with no limitation; 1 = walks with limitation; and 2 = could not walk.

Paw stimulation test

A technician (YN) blinded to the animal groups performed the paw stimulation test. The latency of the hind limb-withdrawal response to radiant heat delivered to the plantar surface was measured before injection of the drug (pre-latency), and after 1 hour, 2 hours, and 7 days (PID 7) post-latency. The tests were repeated 6 times for both the left and right hind paws of each rat and the limb-withdrawal responses were timed. The data were converted to percent maximum possible effect (%MPE), calculated as ([post-latency－pre-latency]/[cut-off time －pre-latency] ×100). We fixed the cut-off (i.e., maximum exposure) time to 20 seconds to prevent thermal injury to the rats' paws.

Tissue preparation

On PID 7 following two functional tests, the animals

were deeply anesthetized with 100 mg/kg of sodium pentobarbital intraperitoneally and perfused transcardially with a fixative solution (2.5% cacodylate-buffered glutaraldehyde) for histological examination. After fixation, the lumbar spinal cord with the anterior and posterior roots and the cauda equina were removed *en bloc* and dissected into 4 samples (A－D) (Figure 1): (A) A transverse section with both roots of L2, (B) posterior and (C) anterior roots proximal to the dorsal ganglion, and (D) cauda equina nerves for light and electron microscopic examinations, as described in our previous studies.[12-15] Tissue samples were stained with Toluidine Blue.

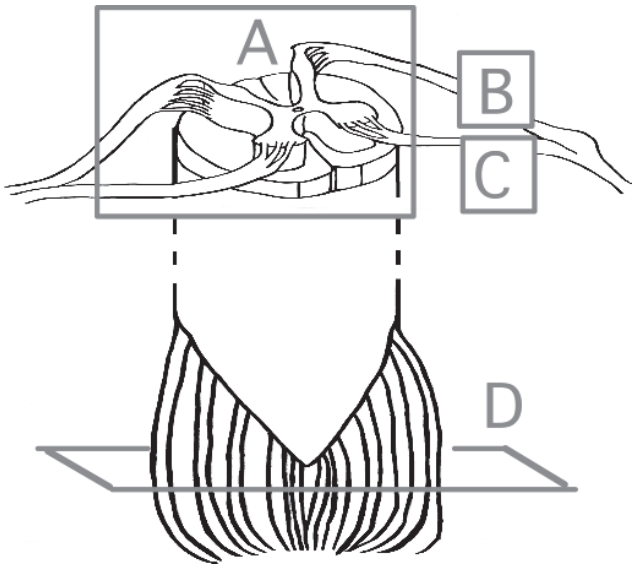

Figure 1. Sample for histological examination

The third lumbar spinal cord with the anterior and posterior roots and the cauda equina were dissected and removed en bloc to prepare 4 samples. (**A**) Third lumbar spinal cord with central portion of both roots. (**B**) Peripheral portion of posterior root. (**C**) Peripheral portion of anterior root. (**D**) Cauda equina.

Statistical analysis

Walking behavior was analyzed by the Kruskal-Wallis test. Values of %MPE were expressed as mean ± SD, and differences in this parameter between each test group and the control (saline solution) group were tested by ANOVA (analysis of variance) followed by the Bonferroni correction. All statistical procedures were performed using StatView software version 4.5 J (Abacus Concept, Berkeley, CA, USA). A P value of <0.05 was considered to indicate a statistically significant difference.

Results

Four rats were excluded from the study because of isotropic events: hind limb palsy, caused by traumatic injury (3 rats); and subarachnoiditis, due to catheterization (1 rat), confirmed by histology. The remaining 35 rats were divided into: the control group (saline, n = 5); the fentanyl groups (0.05 mg/ml [0.05 F], n = 5; 0.5 mg/ml [0.5 F] n = 5; 1 mg/ml [1 F], n = 5); and the bupivacaine groups (5 mg/ml [5 B], n = 5; 25 mg/ml [25 B], n = 5; 50 mg/ml [50 B], n = 5).

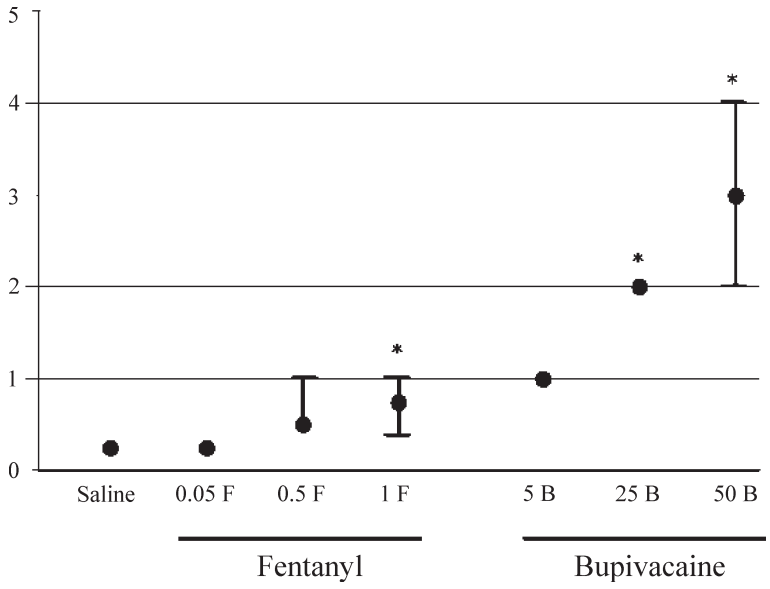

Figure 2. Recovery time to normal ambulation after intrathecal drug injection

F, fentanyl group (mg/ml); B, bupivacaine group (mg/ml). Data are expressed as median and range (minimum-maximum). *P < 0.05 vs. saline

Neurofunctional deficits

Recovery time to ambulation: While all of the rats in both the control and the 0.05-F groups showed complete recovery within 15 minutes after intrathecal injection, those of the 5-B group recovered within 1 hour post-injection. Rats injected with more than 0.5 F or 25 B showed a significantly longer recovery than did the control rats and dose-dependent recovery (Figure 2). No rats required intubation. All rats had recovered to a score of 0 at 15 minutes in the control and the 0.05-F groups, 0.5 hour (0.5－1 hour) in the 0.5-F group, 0.75 hour (0.5－1 hour) in the 1-F group, 1 hour in the 5-B group, 2 hours in the 25-B group, 3 hours (2－4 hours) in the 50-B group, respectively, as expressed by the mean (range) values. When compared with the control, 1 F, 25 B, and 50 B were significantly longer in recovery time.

Paw stimulation test: The effect of drug injections on the sensory threshold expressed as %MPE are shown in Figure 3. In the fentanyl groups (Figure 3A), the sensory threshold increased in a concentration-dependent manner at 1 hour after the injection, and was significantly higher in the 1-F group than that in the control rats ($P = 0.002$). Two hours and then again at 1 week after the injection, there were significant differences among the groups. In the bupivacaine groups (Figure 3B), the sensory threshold was significantly increased in the 25-B and 50-B groups

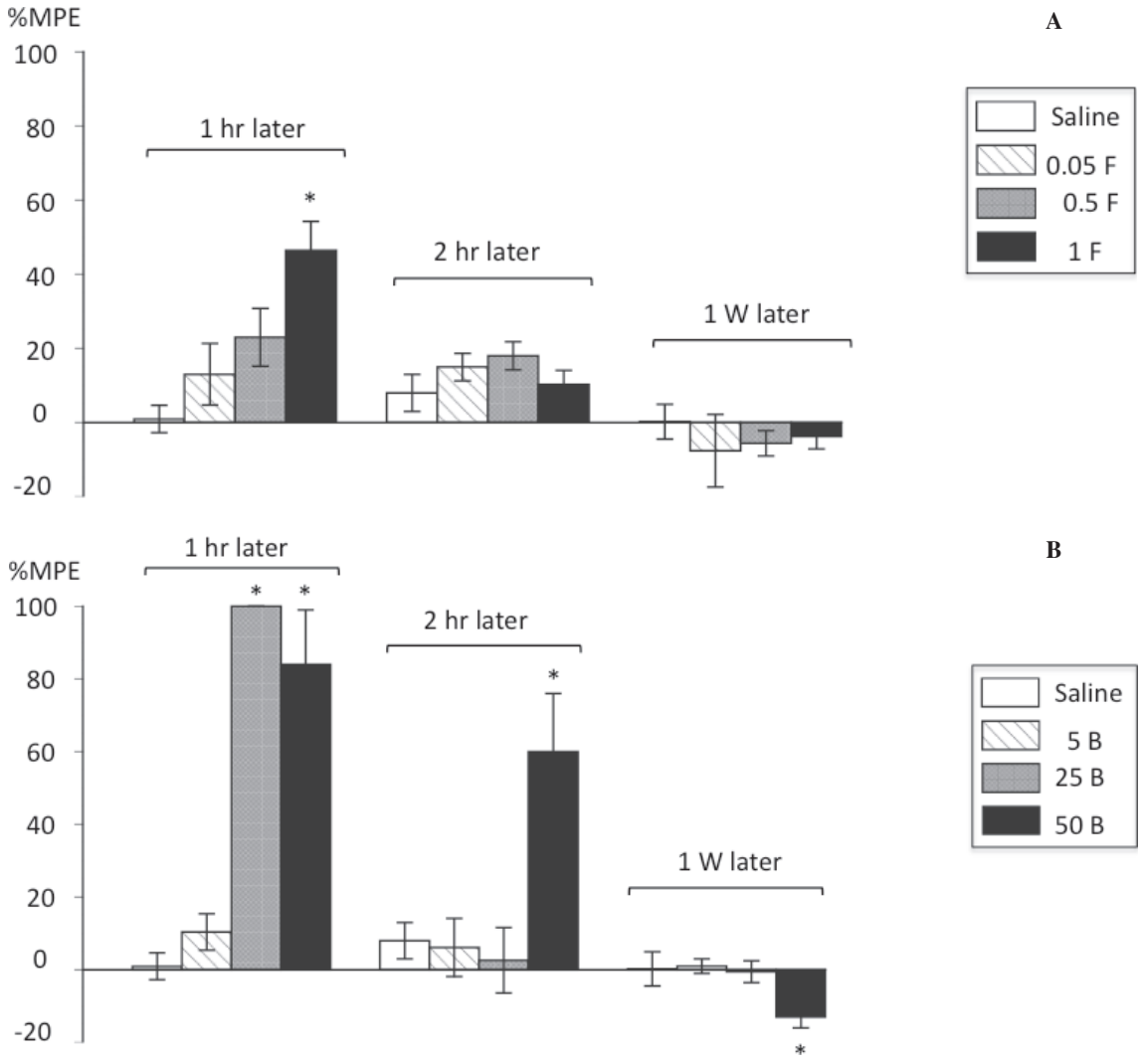

Figure 3. Changes in percent maximum possible effect (%MPE)

In fentanyl groups (**A**), the sensory threshold increased in a concentration-dependent manner at 1 hour after injection and was significantly higher in 1 F than that in the control. However, 2 hours and 1 week after the injection, there are not significant differences among the groups. In the bupivacaine group (**B**), the sensory threshold was significantly higher in 25 B and 50 B after 1 hour, 50 B after 2 hours after the injection, while 1 week after the injection, the sensory threshold was significantly lower than that in the control.

Data are mean ± SD. *$P < 0.05$ vs. saline

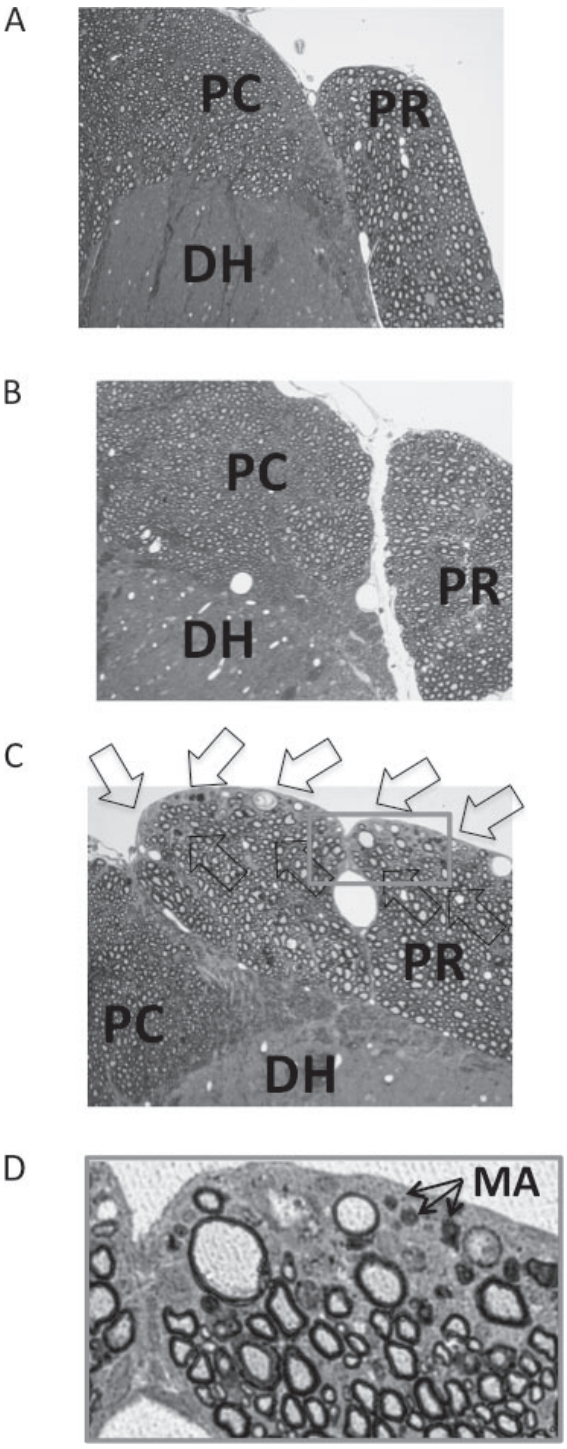

Figure 4. Light microscopy findings of posterior root and posterior white matter

(**A**) Saline, (**B**) 1,000 μg/ml fentanyl (1 F), (**C**) 50 mg/ml bupivacaine (50 B) (×200). Mild histological abnormalities were noted in the margins of the posterior root (PR) (white arrows). (**D**) Magnification of the damaged area in the red square in (**C**) indicating infiltrated macrophage (MA) (gray area) with destruction of the myelin sheaths and/or axons (×400). Tissue samples were stained with Toluidine Blue.
PC, posterior column; DH, dorsal horn.

at 1 hour, and in the 50-B group at 2 hours after the injection, compared with that in the control. One week after the injection, %MPE of the 50-B group was significantly lower than that in the control.

Histopathological assessment of neurotoxicity

Histological abnormality represented a lesion containing infiltrated macrophages with destruction of the myelin sheaths and/or axons. No histological abnormalities were observed in the spinal cord, anterior roots, and cauda equina nerves in the control group, the 5-B and 25-B groups, or any of the fentanyl groups (0.05 F, 0.5 F, 1 F). However, 3 rats in the 50-B group showed histological abnormalities in the central portion of the posterior roots and the lateral area of the posterior column in 1 of 3 rats in the spinal cord (sample A) (Figure 4A). Other areas including the median area of the posterior column, the dorsal horn, and the anterior horn in the spinal cord (sample A), the peripheral portion of the posterior root (sample B) and the anterior root just above the dorsal ganglion (sample C), and the cauda equina nerves (sample D) were intact. In the damaged areas of the posterior root and the posterior column within the spinal cord, posterior root damage was more severe than was that to the posterior column. Moreover, histological damage of the posterior column was observed only when posterior root damage was severe. There was no significant difference in the incidence of lesions to the proximal portion of the posterior or fasciculus cuneatus of the posterior root compared to the control (Table 1). These light microscopic findings of the posterior root and fasciculus cuneatus damage observed in the 50-B group were similar to those described in previous reports.[12,13]

Table 1. Incidence of histological lesions in each area

Group	Posterior root		Posterior column		DH	AH
	Proximal	Distal	LPC	MPC		
Saline (n = 5)	0	0	0	0	0	0
50 F (n = 5)	0	0	0	0	0	
500 F (n = 5)	0	0	0	0	0	0
1000 F (n = 5)	0	0	0	0	0	0
5 B (n = 5)	0	0	0	0	0	0
25 B (n = 5)	0	0	0	0	0	0
50 B (n = 5)	3 (60%)	0	1 (20%)	0	0	0

Proximal, posterior root at entry into the spinal cord; Distal, peripheral portion of the posterior root just proximal to the dorsal ganglion; LPC, lateral area of the posterior column; MPC, medial area of the posterior column; DH, dorsal horn; AH, anterior horn; F, fentanyl group; B, bupivacaine group

We previously found that neurotoxicity of bupivacaine commences from the posterior root just at entry into the posterior column (entry zone), and extends to the lateral area of the posterior column by axonal degeneration because those lesions appeared to be axonal dominant by electron microscopic study.[12,13] Microscopic examination showed axonal degeneration, with intact myelin sheath in the mild injury area treated with bupivacaine. However, degeneration of both the axon and myelin sheath was noted in areas of severe injury. Therefore, we concluded that axons of the posterior roots are the main targets of the neurotoxicity of bupivacaine.

A white neomembrane was occasionally observed around the catheter or catheter tip. Infiltration of macrophages, therefore, seemed a reaction to tissue degeneration following catheter insertion. There were no significant differences in the incidences or neomembrane formation between the groups (Table 2).

Side effects

Table 3 lists the incidences of apnea and rigidity in each group. Apnea was defined as cessation of breathing for more than 10 seconds, and was not observed in any of the rats in the control or bupivacaine groups. One rat in the 0.05-F group, 2 rats in the 0.5-F group, and 3 rats in the 1-F group exhibited apnea for 1 minute, 10 minutes, and 10 minutes, respectively. All the rats received mask ventilation during the apnea. None of the rats required tracheal intubation. The mean apnea duration was 1 minute in the 0.05-F group, 6 minutes (0－15 minutes) in the 0.5-F group, and 8 minutes (0－15 minutes) in the 1-F group.

The incidences of apnea after intrathecal 1 mg/ml fentanyl (P = 0.001) and muscle rigidity after intrathecal 0.5 mg/ml (P = 0.04) and 1 mg/ml fentanyl (P = 0.001) administration were significantly higher than those after saline or bupivacaine administration (P < 0.05) (Table 3). Rigidity was only observed in the 0.5-F and 1-F groups: in 3 of 5 rats approximately 5 minutes after the injection, and in all 5 rats approximately 15 minutes after the injection, respectively. The rigidity first appeared in the tail, then progressed systemically. The grade of rigidity was milder in the 0.5-F group than that in the 1-F group. Although tail rigidity was still observed about 20 minutes after the 0.5 mg/ml fentanyl injection, and 40 minutes after the 1 mg/ml fentanyl injection, the rats started to walk slowly. Thirty minutes after the 0.5 mg/ml fentanyl injection, and 1 hour after the 1 mg/ml fentanyl injection, the walking behavior showed almost complete recovery to normality.

Table 2. Incidence of neomembrane formation around catheter

Group	Rate of neomembrane
Saline (n = 5)	1 (20%)
0.05 F (n = 5)	0
0.5 F (n = 5)	2 (40%)
1 F (n = 5)	1 (20%)
5 B (n = 5)	1 (20%)
25 B (n = 5)	0
50 B (n = 5)	1 (20%)

Table 3. Incidences of side effects

Group	Apnea	Muscle rigidity
Saline (n = 5)	0	0
0.05 F (n = 5)	1 (20%)	0
0.5 F (n = 5)	2 (40%)	3 (60%)*
1 F (n = 5)	5 (100%)*	5 (100%)*
5 B (n = 5)	0	0
25 B (n = 5)	0	0
50 B (n = 5)	0	0

Apnea after intrathecal 1 mg/ml fentanyl (P = 0.001); muscle rigidity after intrathecal 0.5 mg/ml (P = 0.04); 1 mg/ml fentanyl (P = 0.001); after saline or bupivacaine administration (P < 0.05)
*P < 0.05 vs. saline

Discussion

We have previously reported[12-15] that the similarity of neuropathological changes was induced by intrathecal injection of tetracaine,[15] lidocaine,[12,13] mepivacaine,[14] prilocaine,[14] bupivacaine,[12,13] levobupivacaine,[13] and ropivacaine.[13] Namely, histological lesions commence from the posterior root at the entry into the posterior column (entry zone), and extend to the posterior column by axonal degeneration. Even after high-dose injection, the anterior horns or anterior roots generally remain intact. Therefore, sensory impairment like TNS after an overdose injection of any these anesthetics appears to be induced by posterior root damage, which, in turn, has the potential of inducing hyperalgesia.[16,17] Hyperalgesic symptoms such as radiating irritation are also characteristic of TNS.[18] Opioids have also been reported to induce hyperalgesia in animals[19,20] and humans.[21,22] Therefore, we suspected that intrathecal fentanyl neurotoxicity could cause posterior root injury as can local anesthetics. However, the present study demonstrated that unlike bupivacaine, fentanyl induces no evident histological abnormalities in

the spinal cord or nerves, and no irreversible sensory or motor disorders; although fentanyl did induce dose-dependent side effects, such as respiratory depression and muscle rigidity.

In the present study, the sensory threshold increased dose-dependently after intrathecal fentanyl, and increased significantly at 1 hour after a 1-F injection, indicating fentanyl's analgesic effects (Figure 3A). However, 1 week after injection, a significant difference in the sensory threshold was not detected with 1 F but was with 5 B (Figure 3B). This decrease of sensory threshold induced by 5 B appears to be an irritant sensation caused by posterior root damage.

Intrathecal fentanyl neurotoxicity is very rare. In other opioids, histological lesions of the spinal cord itself have been reported although various degrees of inflammation secondary to the catheter were also observed. Sabbe et al.[23] reported that no histological abnormality was seen in the dog spinal cord after intrathecal injection of morphine (0.5 or 5 mg/0.5 ml), sufentanil (5, 25, or 50 μg/0.5 ml), or alfentanil (40 or 400 mg/0.5 ml) for 28 days. Similarly, no histological damage was observed in the cat spinal cord after intrathecal sufentanil (30 μg) or alfentanil (300 μg) for 5 days.[24] In those two studies,[23,24] fibrosis was seen in all the animals, and the degree of fibrosis was not related to the test drugs or saline. On the other hand, Rawal et al.[25] injected 1.5 (small dose) or 7.5 (large dose) μg/kg sufentanil into the sheep subarachnoid space every 6 hours for 3 days. Administration of a large dose caused histological changes in the spinal cord, such as suppurative meningitis and myelitis, as well as spongiosis and chromatolysis. However, neural changes, such as spongiosis, chromatolysis, and axonal changes, were mild, while inflammatory changes such as acute suppurative meningitis were severe. Therefore, such histological lesions of the spinal cord are likely to be induced mainly by inflammation, the spread of which can lead to impairment of neuronal tissue. Therefore, it is conceivable that sufentanil itself does not induce spinal cord damage.

Moreover, even though long-term catheter implantation itself does not induce histological damage to the spinal cord, the inflammatory mass formed around the catheter can theoretically induce spinal cord damage. Granulomas are reported to induce neurological injury by compression of the spinal cord.[25] The incidence of granulomas seems to be higher in animal models than that in humans.[26,27] It is reported that the cumulative risk of developing an inflammatory mass in humans is 0.04% over a 1-year period.[26] In comparison, a more recent experimental study reported that 3 of 3 dogs developed inflammatory masses after only 1 month of catheter implantation.[27] The reason for the different reaction is that in small animals the catheter occupies a relatively larger space in the subarachnoid cavity, which leads to deterioration of cerebrospinal fluid flow and increased contact time and area of the spinal cord and the catheter. These situations likely exacerbate the inflammation.

Although it is not clear whether the effect of inflammation differs according to the type of opioids, Allen et al.[28] demonstrated that all dogs injected with intrathecal morphine, hydromorphine, and methadone showed granulomas, while dogs injected with intrathecal saline or fentanyl did not develop granulomas. In the present study, the inflammatory changes in the rats were mild or nonexistent for fentanyl as well as saline, even at about 40 times the clinical concentration (1 mg/ml vs. 0.025 mg). In the Allen et al.[28] study, the incidence of granuloma formation increased with increased fentanyl concentration. If fentanyl can induce inflammation, granuloma formation would be concentration-dependent. Therefore, these results in the present study suggest that fentanyl does not easily induce spinal cord damage or granulomas.

Histological damage was not induced by fentanyl even when applied at approximately 40 times the clinical concentration (1 F), while posterior root damage was induced by bupivacaine with only 10 times the clinical concentration (0.5 B). Because bupivacaine-induced lesions in the posterior root and posterior column were not accompanied by inflammation, it is possible that the damage appears to be induced by the drug neurotoxicity itself. Based on these results, a mixed solution of the clinical concentrations of fentanyl and bupivacaine would be less likely to increase the frequency of TNS, but rather reduce bupivacaine neurotoxicity by allowing a reduction in the total dose of bupivacaine.

References

1. Ben-David B, Solomon E, Levin H, et al. Intrathecal fentanyl with small-dose dilute bupivacaine: better anesthesia without prolonging recovery. *Anesth Analg* 1997; 85: 560-5.
2. Wang C, Chakrabarti MK, Whitwam JG. Specific enhancement by fentanyl of the effects of intrathecal bupivacaine on nociceptive afferent but not on sympathetic efferent pathways in dogs. *Anesthesiology* 1993; 79: 766-73.
3. Cuvas O, Basar H, Yeygel A, et al. Spinal anesthesia for transurethral resection operations: levobupivacaine with or without fentanyl. *Middle East J Anaesthesiol* 2010; 20: 547-52.

4. Ben-David B, Frankel R, Arzumonov T, et al. Minidose bupivacaine-fentanyl spinal anesthesia for surgical repair of hip fracture in aged. *Anesthesiology* 2000; 92: 6-10.
5. de Santiago J, Santos-Yglesias J, Giron J, et al. Low-dose 3 mg levobupivacaine plus 10 microg fentanyl selective spinal anesthesia for gynecological outpatient laparoscopy. *Anesth Analg* 2009: 109: 1456-61.
6. Gunaydin B, Tan ED. Intrathecal hyperbaric or isobaric bupivacaine and ropivacaine with fentanyl for elective caesarean section. *J Matern Fetal Neonatal Med* 2010; 23: 1418-6.
7. Black AS, Newcombe GN, Plummer JL, et al. Spinal anaesthesia for ambulatory arthroscopic surgery of the knee: a comparison of low-dose prilocaine and fentanyl with bupivacaine and fentanyl. *Br J Anaesth* 2011; 106: 183-8.
8. Hampl KF, Schneider MC, Thorin D, et al. Hyperosmolarity does not contribute to transient radicular irritation after spinal anesthesia with hyperbaric 5% lidocaine. *Reg Anesth* 1995; 20: 363-8.
9. Hampl KF, Heinzmann-Wiedmer S, Luginbuehl I, et al. Transient neurologic symptoms after spinal anaesthesia: a lower incidence with prilocaine and bupivacaine than with lidocaine. *Anesthesiology* 1998; 88: 629-33.
10. Keld DB, Hein L, Dalgaard M, et al. The incidence of transient neurologic symptoms (TNS) after spinal anaesthesia in patients undergoing surgery in the supine position. Hyperbaric lidocaine 5% versus hyperbaric bupivacaine 0.5%. *Acta Anaesthesiol Scand* 2000; 44: 285-90.
11. Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 1976; 17: 1031-6.
12. Takenami T, Yagishita S, Murase S, et al. Neurotoxicity of intrathecally administered bupivacaine involves the posterior roots/posterior white matter and is milder than lidocaine in rats. *Reg Anesth Pain Med* 2005; 30: 464-72.
13. Takenami T, Yagishita S, Arai M, et al. Neurotoxicity of intrathecally administered tetracaine commences at the posterior roots near entry into the spinal cord. *Reg Anesth Pain Med* 2000; 25: 372-429.
14. Takenami T, Yagishita S, Nara Y, et al. Intrathecal mepivacaine and prilocaine are less neurotoxic than lidocaine in a rat intrathecal model. *Reg Anesth Pain Med* 2004; 29: 446-53.
15. Takenami T, Wang G, Nara Y, et al. Intrathecally administered ropivacaine is less neurotoxic than procaine, bupivacaine, and levobupivacaine in a rat spinal model. *Can J Anaesth* 2012; 59: 456-65.
16. Huang C, Zou W, Lee K, et al. Different symptoms of neuropathic pain can be induced by different degrees of compressive force on the C7 dorsal root of rats. *Spine J* 2012; 12: 1154-60.
17. Hunt JL, Winkelstein BA, Rutkowski MD, et al. Repeated injury to the lumbar nerve roots produces enhanced mechanical allodynia and persistent spinal neuroinflammation. *Spine* 2001; 26: 2073-9.
18. Pollock JE. Neurotoxicity of intrathecal local anesthetics and transient neurological symptoms. *Best Pract Res Clin Anaesthesiol* 2003; 17: 471-84.
19. Komatsu T, Sakurada S, Katsuyama S, et al. Mechanism of allodynia evoked intrathecal morphine-3-glucuronide in mice. *Int Rev Neurobiol* 2009; 85: 207-19.
20. Van Elstraete AC, Sitbon P, Mazoit JX, et al. Gabapentin prevents delayed and long-lasting hyperalgesia induced by fentanyl in rats. *Anesthesiology* 2008; 108: 484-94.
21. Ossipov MH, Lai J, King T, et al. Antinociceptive and nociceptive actions of opioids. J Neurobiol 2004; 61: 126-48.
22. Devulder J. Hyperalgesia induced by high-dose intrathecal sufentanil on neuropathic pain. *J Neurosurg Anesthesiol* 1997; 9: 146-8.
23. Sabbe MB, Grafe MR, Mjanger E, et al. Spinal delivery of sufentanil, alfentanil, and morphine in dogs. Physiologic and toxicologic investigations. *Anesthesiology* 1994; 81: 899-920.
24. Yaksh, TL, Noueihed RY, Durant PA. Studies of the pharmacology and pathology of intrathecally administered 4-anilinopiperidine analogues and morphine in the rat and cat. *Anesthesiology* 1986; 64: 54-66.
25. Rawal N, Nuutinen L, Raj PP, et al. Behavioral and histopathologic effects following intrathecal administration of butorphanol, sufentanil, and nalbuphine in sheep. *Anesthesiology* 1991; 75: 1025-34.
26. North RB, Cutchis PN, Epstein JA, et al. Spinal cord compression complicating subarachnoid infusion of morphine: case report and laboratory experience. *Neurosurgery* 1991; 29: 778-84.
27. Yaksh TL, Hassenbusch S, Burchiel K, et al. Inflammatory masses associated with intrathecal drug infusion: a review of preclinical evidence and human data. *Pain Med* 2002; 3: 300-12.
28. Allen JW, Horais KA, Tozier NA, et al. Opiate pharmacology of intrathecal granulomas. *Anesthesiology* 2006; 105: 590-8.