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 Okamoto¹

¹Department of Anesthesiology, Kitasato University School of Medicine  
²Department of Pathology, Kanagawa Rehabilitation Center, Kanagawa  
³Department 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...
Takenami, et al.

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. Tissue samples were stained with Toluidine Blue.

**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 1. Sample for histological examination](image)

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.

![Figure 2. Recovery time to normal ambulation after intrathecal drug injection](image)

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
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

![Figure 4. Light microscopy findings of posterior root and posterior white matter](image)

(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.

**Table 1. Incidence of histological lesions in each area**

<table>
<thead>
<tr>
<th>Group</th>
<th>Posterior root</th>
<th>Posterior column</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
</tr>
<tr>
<td>Saline (n = 5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50 F (n = 5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>500 F (n = 5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1000 F (n = 5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 B (n = 5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25 B (n = 5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>50 B (n = 5)</td>
<td>3 (60%)</td>
<td>1 (20%)</td>
</tr>
</tbody>
</table>

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. 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.

**Discussion**

We have previously reported that the similarity of neuropathological changes was induced by intrathecal injection of tetracaine, lidocaine, mepivacaine, prilocaine, bupivacaine, and ropivacaine. 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 of these anesthetics appears to be induced by posterior root damage, which, in turn, has the potential of inducing hyperalgesia. Hyperalgesic symptoms such as radiating irritation are also characteristic of TNS. Opioids have also been reported to induce hyperalgesia in animals and humans. 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. 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. In those two studies, 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. 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. In those two studies, fibrosis was seen in all the animals, and the degree of fibrosis was not related to the test drugs or saline.

Inflammation differs according to the type of opioids, 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


