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COMPARISON OF THE ANALGESIC POTENCY BETWEEN INTRATHECAL ROPIVACAINE AND BUPIVACAINE IN RATS

Tomoki Nishiyama MD, PhD*

Department of Emergency Medicine (Anesthesiology), Maruyama Memorial General Hospital, 2-10-5, Hon-cho, Iwatsuki-ku, Saitama-shi, Saitama, 339-8521, Japan.
This study was done at the University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, Japan.

***Corresponding Author: Dr. Tomoki Nishiyama MD, PhD**
Department of Emergency Medicine (Anesthesiology), Maruyama Memorial General Hospital, 2-10-5, Hon-cho, Iwatsuki-ku, Saitama-shi, Saitama, 339-8521, Japan. **DOI: https://doi.org/10.17605/OSF.IO/7QJGV**

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ABSTRACT

Background: There is no study to compare analgesic effects of ropivacaine and bupivacaine on different kinds of pain. We compared the effects of intrathecal ropivacaine and bupivacaine for different kinds of pain using acute thermal and formalin induced pain models of rats. **Methods:** Male Sprague-Dawley rats implanted with lumbar intrathecal catheters were given some different doses of intrathecal ropivacaine or bupivacaine, then tail flick test or formalin test was performed. The 50% effective doses (ED50s) were calculated and compared between ropivacaine and bupivacaine. Behavioral side effects were also checked. **Results:** The ED50 of bupivacaine was significantly lower than that of ropivacaine in the tail flick test, but it was significantly higher than that of ropivacaine in both phases of the formalin test. ED50s of both bupivacaine and ropivacaine were lower in both phases of the formalin test than those in the tail flick test. Behavioral side effects were observed only with bupivacaine. **Conclusions:** We suggest that intrathecal analgesia with ropivacaine is better than bupivacaine for inflammatory pain, both acute and chronic phases, but bupivacaine is better for acute thermal pain.

KEYWORDS: Analgesia, Intrathecal, local anesthetic, Ropivacaine, Bupivacaine.

INTRODUCTION

There are many clinical studies to compare analgesic effects of ropivacaine and bupivacaine in spinal anesthesia. Some different results in onset time and potency were seen among the studies whether these agents were used with or without glucose, but duration of sensory and motor block were shorter with ropivacaine than bupivacaine in all studies.[1-5] Therefore, the choice for spinal anesthesia should depend on the duration of surgery. However, in pain management, various kinds of pain could be treated. No studies have been seen to compare the effects of intrathecal ropivacaine and bupivacaine on different pain. This study compared the effects of intrathecal ropivacaine and bupivacaine in different pain stimuli using rat models.

MATERIALS AND METHODS

After obtaining the approval of the Research Committee of the University of Tokyo, male Sprague-Dawley rats (280-300 g; Nippon Bio-Supply, Tokyo, Japan) were implanted with lumbar intrathecal catheters under halothane (2 %) anesthesia. The experiment procedures are the same as our previous study.[6] Briefly, an 8.5 cm polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ) was inserted caudally to the thoracolumbar level in the intrathecal space through atlanto-occipital membrane. The rostral part of the catheter was plugged with a 28-gauge steel wire and put through to the top of the skull. Only rats with normal motor function and behavior and increase in body weight seven days later were used for experiments. After the study, rats were euthanized under halothane 5% and the location of the catheter was confirmed anatomically and the data of the rats with mal location of the catheter was excluded and another rat was added to fill the number of each group.

Drug preparation

Bupivacaine and ropivacaine (Sigma, St. Louis, MO) were dissolved in normal saline to get each target concentration in 10μl. For bupivacaine, 1, 10, 30, and 100 μg were used in both tail flick test and formalin test. For ropivacaine, 1, 3, 10, 30, and 100 μg in the tail flick test and 0.1, 1, 3, 10, and 30μg in the formalin test were used.

Nociceptive test

According to our previous study,[6] we used the same two well-known methods as nociceptive tests.

Tail-flick test

The tail-flick test was performed with the Tail-Flick Analgesia Meter (MK-330A; Muromachi Kikai Co. Ltd., Tokyo, Japan). Rats were placed in a clear plastic cage with their tails extending through a slot located of the rear of the cage. Thermal stimulation was given by a beam of high intensity light focused on the tail 2 to 3 cm proximal to the end.

The time between the start of the stimulation and tail withdrawal response was measured as a tail-flick latency. The cut-off time in the absence of a response was set to 14 seconds to prevent tissue injury of the tail. The test was done at 5, 10, 15, 30, 60, 90, 120, 180, and 240 minutes after drug injection. The data were shown as the % of maximum possible effect (% MPE): % MPE = (post-drug latency – pre-drug latency at time 0) X 100 / (cut-off time (14 sec) – pre-drug latency at time 0.

Formalin test

The formalin test was performed 10 minutes after intrathecal drug injection. Fifty µL of 5 % formalin was injected subcutaneously into the dorsal surface of the right hind paw with a 30 G needle. Immediately after injection, the rat was placed in an open clear plastic chamber and their flinching or shaking paw response was observed for 60 minutes. The number of flinches was counted for 1 minute. Usually two phases were observed: phase 1, during 0 to 6 minutes after formalin injection; and phase 2, beginning about 10 minutes after injection with the interval of no flinches between both phases.

Side effects

Side effects were examined and judged as present or absent. Agitation was judged as spontaneous irritable movement, vocalization, or both. Allodynia-like behavior was judged as escape, vocalization, or both induced by lightly stroking the flank of the rat with a small probe. The placing or stepping reflex was evoked by drawing the dorsum of either hind paw across the edge of the table. Normal rats try to put the paw ahead into a position to walk. The righting reflex was assessed by placing the rat horizontally with its back on the table. Normally rats twist the body to an upright position immediately. Flaccidity was judged as muscle weakness by putting the forepaw 3 to 5 cm higher than the hind paw. Normal rats will walk up. Pinna or corneal reflex was examined with a paper string. When a string is put into the ear canal or touches the cornea, rats normally shake their heads. Behavioral side effects were checked simultaneously with the tail flick test.

Protocol

Each drug was administered intrathecally and after injection of the drug, the catheter was flushed with normal saline 10 µL to clear the dead space of the catheter. We have already used intrathecal bupivacaine in both tail flick test and formalin test in our previous study.[7]

We used the data of the first 4 rats with bupivacaine in our previous study and add new 4 rats to save the animals. For ropivacaine, each 8 rats were used in each test. The ED50 was obtained using the maximum effects in the tail flick test and the area under the curve of the number of flinches in the formalin test.

Data analysis

The data are shown as mean ± standard deviation or 95% confidential interval (CI). Statistical analysis was performed with the factorial analysis of variance (ANOVA). A p value less than 0.05 was considered to be statistically significant.

RESULTS

Dose dependent increases in the tail flick latency were observed in both bupivacaine (Fig.1) and ropivacaine (Fig.2).

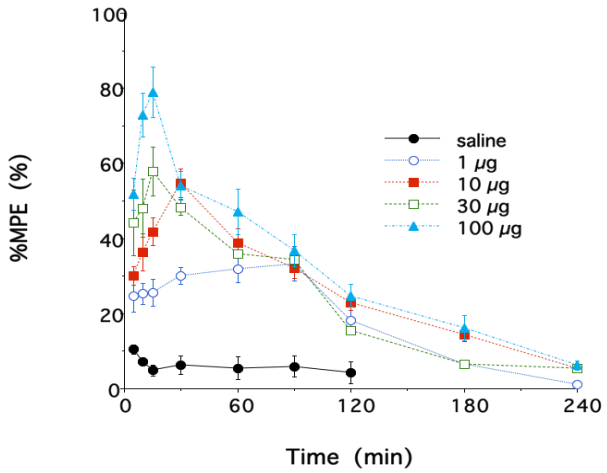

Figure 1: Tail flick test with intrathecal bupivacaine.
%MPE, % of maximum possible effect; Bars indicate standard deviation.

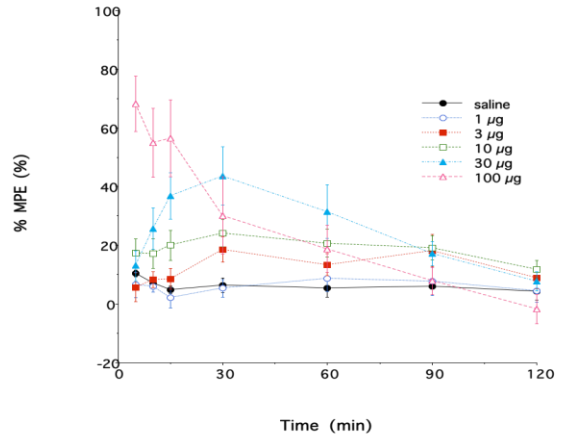

Figure 2: Tail flick test with intrathecal ropivacaine.
%MPE, % of maximum possible effect; Bars indicate standard deviation.

Paw flinch responses to formalin administration decreased dose dependently in both bupivacaine (Fig.3) and ropivacaine (Fig.4).

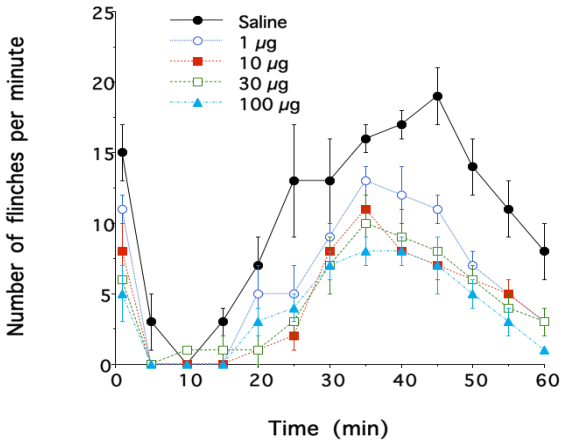

Figure 3: Formalin test with intrathecal bupivacaine.
Bars indicate standard deviation

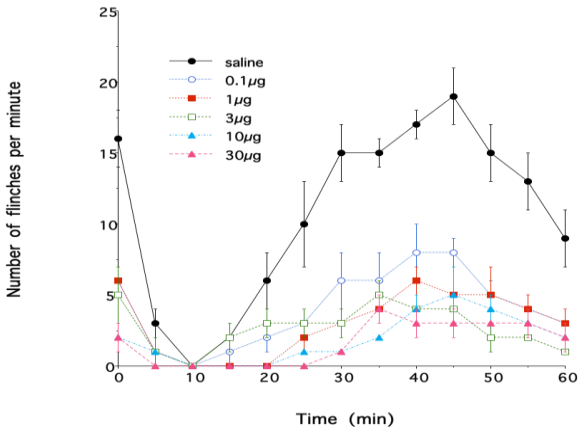

Figure 4: Formalin test with intrathecal ropivacaine.
Bars indicate standard deviation.

The ED50 of bupivacaine was significantly lower than that of ropivacaine in the tail flick test (Table 1), but it was significantly higher than that of ropivacaine in both phases of the formalin test (Table 1).

Table 1: ED50.

	Tail flick	Formalin phase 1	Formalin phase 2
Ropivacaine (µg)	30.5 (20.8-44.6)	0.05 (0.01-0.12)	0.005 (0.0002-0.01)
Bupivacaine (µg)	7.0* (3.7-13.1)	5.5* (1.9-15.4)	3.3* (0.1-12.0)

Mean and 95% confidence interval in the parenthesis
*: $P < 0.05$ vs. Ropivacaine

Side effects were observed only with bupivacaine (Table 2).

Table 2: Side effects.

Dose of bupivacaine	1 µg	10 µg	30 µg	100 µg
Agitation	1	0	0	0
Allodynia	1	1	0	1
Ambulation	0	0	2	4
Asymmetry	0	0	2	3
Flaccidity	0	0	2	4
Disturbance of righting reflex	1	1	2	4
Disturbance of stepping reflex	1	0	2	4

Number of rats who showed each side effect was shown. Total number of tested was 8.

DISCUSSION

The present study showed that intrathecal ropivacaine was less potent than bupivacaine in the tail flick test, but more potent in both phases of the formalin test. Only bupivacaine induced side effects.

In intrathecal administration, onset time was longer with ropivacaine than bupivacaine when used as glucose contained solution,[1,4] but similar as glucose free solution[2] in clinical studies. Our study used glucose free solution, but could not evaluate onset time.

Ropivacaine had shorter duration of sensory and motor blocks than bupivacaine in spinal anesthesia whether combined with or without glucose.[1-4,8] In our study, intrathecal bupivacaine showed longer duration of action than ropivacaine in the tail flick test, but we could not determine the duration in the formalin test because we stopped measurement in 60 minutes in both ropivacaine and bupivacaine and number of flinches did not reach zero in both bupivacaine and ropivacaine.

Ropivacaine has lower lipid solubility than bupivacaine,[5] then has smaller penetration of large myelinated motor fibers,[8] which induces decreased intensity and shorter duration of motor block. Ropivacaine constricts and bupivacaine dilates pial vessels of the spinal cord in a concentration dependent.[9]

Therefore, intrathecal ropivacaine decreased spinal cord blood flow.[10] This might have some roles in shorter duration of the block with ropivacaine.

In our study, only bupivacaine showed side effects. Agitation and allodynia might be in some part by strong sensory blockade. Symmetry, flaccidity, and disturbance of righting, placing and stepping reflex might be due to motor disturbance. These suggested intrathecal bupivacaine had stronger sensory and motor block than ropivacaine.

We could not find any studies to compare intrathecal ropivacaine and bupivacaine in the tail flick test and formalin test. Kang et al.[11] reported that ED50s of intrathecal bupivacaine in rats for phase 1 and 2 of the formalin test were 11.97 µg (5.34 – 25.76 µg) and 9.06 µg (5.75 – 13.64 µg), respectively. Their ED50s were larger than our data, which might be due to different environment and technique of experiment. However, they are consistent with the point that intrathecal bupivacaine was more effective for phase 2 than for phase 1 of the formalin test. This is the same with ropivacaine in our present study.

The phase 1 is caused by direct stimulation of peripheral nociceptors, and phase 2 is related to inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord.[12]

Therefore, both intrathecal ropivacaine and bupivacaine had more effective for inflammatory facilitated pain than for chemical induced acute pain.

Intrathecal ropivacaine had weaker analgesic effect for acute thermal pain than bupivacaine in our present study.

Bupivacaine decreased high-voltage-activated calcium current, which plays a great role in regulating

neurotransmitter release, dose-dependently. In contrast, ropivacaine increased high-voltage-activated calcium current at lower concentrations, but decreased it at higher concentrations.[13] These might be one of the reasons why high dose of ropivacaine were necessary for acute thermal pain.

Intrathecal bupivacaine had almost similar potency in analgesia for acute thermal pain and chemical induced acute pain, and less potent for inflammatory facilitated pain, but ropivacaine had significantly potent in the order of inflammatory facilitated pain > chemical induced acute pain > acute thermal pain. The reason of these differences was not known from our study. Therefore, further study is necessary.

From our results, we suggest that ropivacaine is better than bupivacaine for chemical or inflammatory pain, both acute and chronic phases, but bupivacaine is better for acute thermal pain when administered intrathecally.

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