Memantine elicits spinal blockades of motor function, proprioception, and nociception in rats

Running Head: Spinal anesthesia with memantine

Yu-Wen Chen a,b, Chong-Chi Chiu c,d, Kuo-Sheng Liu e, Ching-Hsia Hung f,g,*, Jhi-Joung Wang b

a Department of Physical Therapy & Graduate Institute of Rehabilitation Science, College of Health Care, China Medical University, Taichung, Taiwan
b Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan
c Department of General Surgery, Chi Mei Medical Center, Tainan and Liouying, Taiwan
d Department of Electrical Engineering, Southern Taiwan University of Science and Technology, Tainan, Taiwan
e Department of Pharmacy, Chia Nan University of Pharmacy and Science, Tainan, Taiwan
f Department of Physical Therapy, College of Medicine, National Cheng Kung University, Tainan, Taiwan
g Institute of Allied Health Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/fcp.12142
This article is protected by copyright. All rights reserved.
ABSTRACT
Although memantine blocks sodium currents and produces local skin anesthesia, spinal anesthesia with memantine is unknown. The purpose of the study was to evaluate the local anesthetic effect of memantine in spinal anesthesia and its comparison with a widely-used local anesthetic lidocaine. After intrathecally injecting the rats with five doses of each drug, the dose—response curves of memantine and lidocaine were constructed. The potencies of the drugs and durations of spinal anesthetic effects on motor function, proprioception, and nociception were compared to those of lidocaine. We showed that memantine produced dose-dependent spinal blockades in motor function, proprioception, and nociception. On a 50% effective dose (ED_{50}) basis, the rank of potency was lidocaine greater than memantine (P < 0.05 for the differences). At the equipotent doses (ED_{25}, ED_{50}, ED_{75}), the block duration produced by memantine was longer than that produced by lidocaine (P < 0.05 for the differences). Memantine, but not lidocaine, displayed more sensory/nociceptive block than motor block. The preclinical data demonstrated that memantine is less potent than lidocaine, whereas memantine produces longer duration of spinal anesthesia than lidocaine. Memantine shows a more sensory-selective action over...
motor blockade.

Key Words: memantine, lidocaine, spinal block, motor function, proprioception, nociception

INTRODUCTION

Memantine is the first drug for the treatment of Alzheimer's disease, and its therapeutic utility is linked to the target for drug action on the glutamatergic system through blocking N-methyl-D-aspartate glutamate receptors [1, 2]. Because memantine reversibly suppressed tetrodotoxin (TTX)-resistant sodium currents [3], it produced a local anesthetic effect on infiltrative cutaneous (peripheral) analgesia [4]. Furthermore, it has been shown that intravenous equianalgesic dose of memantine is better tolerated to cause central nervous system and cardiovascular system toxicity than bupivacaine [5]. In addition, both epinephrine [6] and clonidine [7] increased the depth and duration of infiltrative cutaneous analgesia produced by memantine in rats.

Local anesthetics are commonly administered intrathecally for various procedures or pathologies. Additionally, spinal anesthesia is a relatively simple procedure, which brings acceptable surgical conditions through the injection of a small amount of the local anesthetic solution with easy landmarks, supporting a wide
popularity to the practice [8]. However, to the best of our knowledge, no study of spinal (central) anesthesia following intrathecal injections of memantine has been reported to date. The aim of the study was to evaluate the spinal anesthetic effects on motor function, proprioception, and nociception after intrathecal injections of memantine in rats. Lidocaine, the most commonly used local anesthetic, was used as a control group.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (300 g to 350 g) were purchased from National Cheng Kung University (Tainan, Taiwan) and kept in the animal housing facilities at the same University, with controlled humidity (approximately 50% relative humidity), room temperature (22°C), and a 12-hour (6:00 AM to 6:00 PM) light/dark cycle. All animal care and experimental procedures were approved by the Institutional Animal Care and Use Committee of National Cheng Kung University on Animals and were consonant with the international guidelines for the use of laboratory animals. Memantine hydrochloride and lidocaine hydrochloride monohydrate were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All agents in stock were freshly prepared in 5% dextrose as solution before intrathecal injections.
Spinal anesthesia: intrathecal injection

Lumbar puncture was performed on conscious rats according to the previous studies [9, 10]. In brief, a 27-gauge needle attached to a 50-µl syringe (Hamilton, Reno, Nevada) was inserted into the mid-line of the L4–L5 intervertebral space until a tail-flick indicated entrance into the spinal space. Twenty-five microliters of the drug solution were injected while the animals were observed for the development of spinal anesthesia, indicated by paralysis of both hind limbs. Rats, which displayed unilateral blockade, were excluded from the study and sacrificed by using an over dose of isoflurane. Furthermore, all animals were injected once in the study intrathecally.

Spinal anesthesia: neurobehavioral assessment

After intrathecal administration, three neurobehavioral examinations, which consisted of motor function, proprioception and nociception, were conducted. The magnitudes of spinal blockades in nociception, proprioception and motor function were described as the percentage of possible effect (% PE). The maximum blockade in a time course of spinal anesthesia with the drug was described as the percentage of maximal possible effect (% MPE). For consistency, one experienced investigator, who was blinded to the treatment groups, was responsible for handling the animals and performing neurobehavioral examinations.
Nociception was examined by the withdrawal reflex or vocalization elicited by pinching a skin fold over each rat's back at 1 cm from the proximal part of the tail, the dorsal part of the mid-tail, and the lateral metatarsus of bilateral hind limbs. Sensory/nociceptive block was graded as 4 (normal or 0% MPE), 3 (25% MPE), 2 (50% MPE), 1 (75% MPE), and 0 (absent or 100% MPE) [11, 12]. At each testing time, only one pinch was performed to each of the four testing sites, and the time interval between two stimuli at two different sites was around 2 s.

Proprioception was based on the resting posture and postural reactions (‘tactile placing’ and ‘hopping’). Hopping response was assessed by lifting the front half of the rat off the ground and lifting one hind limb at a time off the ground so that the rat was standing on just one limb. The rat was then moved laterally, which normally evoked a quick hopping response with the weight-bearing limb in the direction of movement to prevent the rat from falling. A markedly motor deficit displayed a quick but weaker than normal response. Conversely, with a markedly proprioceptive impairment, delayed hopping was followed by greater lateral hops to prevent falling over or, in the case of complete block, no hopping at all. The proprioceptive blockade was graded as 3 (normal or 0% MPE), 2 (slightly impaired), 1 (severely impaired), and 0 (completely impaired or 100% MPE) [11, 13].

Motor function was evaluated by measuring ‘the extensor postural thrust’ of the
left hind limb of the rat. The extensor postural thrust was measured as the gram force, which resisted contacting the platform by one rat heel applied to a digital platform balance (Mettler Toledo, PB 1502-S, Switzerland). A decrease in the force, representing reduced extensor muscle tone, was considered as motor impairment and was presented as a percent of the control force. The pre-injection control value was considered as 0% motor block or 0% MPE. A force less than 20 g (also referred to as the weight of the 'flaccid limb') was interpreted as the absence of extensor postural thrust or a 100% motor block or 100% MPE [14, 15].

**Area under the curves (AUCs), full recovery time, and 50% effective dose (ED$_{50}$)**

The AUCs of spinal anesthesia with drugs were calculated by using Kinetica v 2.0.1 (MicroPharm International, USA). The full recovery time was defined as the interval from drug injection to full recovery (0% MPE). The dose—response curves were constructed following injecting the rats with five different doses of each drug intrathecally. Then, the curves were fitted by using a SAS Nonlinear (NLIN) Procedures (SAS Institute Inc., Carey, NC), while the value of ED$_{50}$, defined as the dose that produced 50% spinal blockades of motor function, proprioception and nociception, were obtained [16, 17]. The ED$_{25}$ or ED$_{75}$ of drugs were constructed by using the same curve-fitting methods (SAS NLIN Procedures) which were used to

This article is protected by copyright. All rights reserved.
derive the ED$_{50}$ [18].

**Experimental groups**

Three specific experiments were performed. In experiment 1, spinal anesthesia with memantine (0.75, 1.00, 1.75, 3.00, 5.00 µmol) and lidocaine (0.50, 0.75, 1.00, 2.00, 3.25 µmol) in a dose-dependent manner were tested (n=8 rats for each dose of each drug). In experiment 2, the %MPE, complete blockade time, full recovery time, AUCs of spinal anesthesia with memantine (5 µmol), lidocaine (3.25 µmol), and vehicle (5% dextrose) was assessed (n=8 rats for each dose of each drug). In experiment 3, the duration of memantine in spinal anesthesia was compared with that of lidocaine (n=8 rats for each dose of each drug) at an equipotent basis (ED$_{25}$, ED$_{50}$ and ED$_{75}$).

**Statistical analysis**

Values are expressed as means ± S.E.M. or ED$_{50}$ values with 95% confidence interval (95% CI). Data were analyzed by either 1-way (experiments 1 and 2) or 2-way (experiment 3) analysis of variance (ANOVA) followed by pairwise Tukey's honest significance difference (HSD) test. A statistical software, SPSS for Windows (version 17.0, SPSS, Inc, Chicago, IL, USA), was used, and a $P$ value less than 0.05 was
considered statistically significant.

RESULTS

Spinal anesthesia with memantine and lidocaine in a dosage-dependent fashion

Memantine, as well as the local anesthetic lidocaine produced a dose-dependent local anesthetic effect in spinal anesthesia in rats (Fig. 1). The ED$_{25}$, ED$_{50}$, and ED$_{75}$ of memantine and lidocaine are shown in Table 1. On the ED$_{50}$ basis, the ranks of potencies in motor function, proprioception, and nociception were lidocaine > memantine ($P < 0.05$; Table 1). Moreover, the sensory/nociceptive block (ED$_{50}$, Table 1) was greater than the motor block in the memantine group ($P < 0.05$), but not the lidocaine group ($P > 0.05$).

Spinal anesthesia with memantine at 5 μmol and lidocaine at 3.25 μmol

Memantine at 5 μmol exhibited 100%, 100%, and 100% of blockades (% MPE) in motor function, proprioception, and nociception with duration of action of about 48.8, 58.8, and 103.1 min, respectively (Fig. 2 and Table 2). At a given dose of 3.25 μmol, lidocaine elicited 100%, 100%, and 100% of blockades (% MPE) in motor function, proprioception, and nociception with duration of action of about 30.6, 36.6, and 38.8

This article is protected by copyright. All rights reserved.
min, respectively (Fig. 2 and Table 2). Furthermore, intrathecal injection of 5% dextrose (vehicle) produced no spinal anesthesia (Fig. 2).

**The complete blockade time, full recovery time, and AUCs of memantine and lidocaine in spinal anesthesia**

The full recovery time and AUCs of spinal anesthesia with memantine were significantly greater than those of lidocaine \((P < 0.05)\), whereas the complete blockade time between memantine and lidocaine was not significantly different in Table 2. Moreover, memantine also elicited longer duration of sensory blockade than that of motor blockade \((P < 0.01; \text{Table 2})\). At the equipotent doses \((\text{ED}_{25}, \text{ED}_{50}, \text{ED}_{75})\), the spinal blockades of motor function, proprioception and nociception produced by memantine were longer than those produced by lidocaine \((P < 0.05; \text{Fig. 3})\).

**DISCUSSION**

We showed for the first time that intrathecal memantine produced spinal anesthesia in a dose-dependent manner, whereas memantine was less potent than lidocaine. At the equianesthetic doses, the duration of spinal anesthesia with memantine was greater than that of lidocaine. Memantine also displayed more sensory/nociceptive...
block than motor block. In agreement with our previous studies [4-6], memantine produced a local anesthetic effect on infiltrative cutaneous analgesia in rats.

Local anesthetics produced a conduction block of neural impulses by blocking voltage-gated Na\(^+\) channels in the nervous tissues [19]. Because memantine inhibited TTX-resistant sodium currents [3], it produced spinal blockades of motor function, nociception and proprioception. The resulting data suggest that memantine has the characteristics of a local anesthetic. This is in resemblance to our previous study that subcutaneous memantine produced a dose-dependent local anesthetic effect as infiltrative cutaneous analgesia in rats [4-6]. In the present study, memantine and lidocaine produced dose-dependent spinal anesthesia, whereas lidocaine (ED\(_{50}\)) showed almost 1.9-folds higher potency than did memantine in spinal anesthesia. Our findings are in agreement with the report by Brau et al. [3] who demonstrated that the half-maximal blocking concentration (IC\(_{50}\)) of lidocaine was 1.9-folds more potent than memantine for 2-Hz use-dependent block at -70mV on tetrodotoxin-resistant Na\(^+\) currents in rat sensory neurons.

Memantine has been used for the prevention of neuropathic pain in a rat model of spinal nerve ligation (SNL) [20] and for the management of formalin-induced pain behavior [21]. Recent evidence suggests the utility of memantine in the treatment of patients with Fibromyalgia (FM) [22, 23] or subjects with post-mastectomy
neuropathic pain [24]. Here, we revealed that intrathecal memantine produced a completely sensory/nociception block. The action of memantine as a Na\(^+\) channel blocker [3] may contribute the results in preventing patients from painful sensation. In addition, neuronal TTX-resistant Na\(^+\) channels play a critical role in the generation of nociceptive impulses in nerve fibers [25, 26], while memantine has been shown to suppress TTX-resistant sodium currents [3]. In the present experiment, the nociceptive/sensory block of memantine was almost 1.2-folds higher potency (ED\(_{50}\)) than the motor block. We agree that there is a detectable difference between motor vs. sensory blockade; i.e., bupivacaine in general produces more sensory/nociceptive block than motor block [27]. Furthermore, memantine exhibited a longer duration of sensory block than that of motor block (Table 2). Clinically, the local anesthetic agents have commonly been used to produce complete nerve block and not to augment the potency.

Surgery by injection of a long-acting local anesthetic is commonly performed [28, 29]. Spinal anesthesia, a relatively easy technique, carries satisfactory surgical conditions by injecting a small amount of local anesthetic solutions with easy landmarks [8, 30]. There are few cases where ultrashort spinal anesthesia is needed. For the reason, they use lidocaine or 2-chloroprocaine clinically [31, 32]. Consequently, we also evaluated the effects of memantine and lidocaine at the
equianesthetic doses (ED$\text{25}$, ED$\text{50}$, ED$\text{75}$). The resulting data demonstrated that the duration caused by memantine in spinal anesthesia was greater than that caused by lidocaine on an equipotent basis (Fig. 3). Furthermore, the duration of spinal anesthesia with memantine (5 μmol) was longer than that of lidocaine (3.25 μmol) (Table 2). The application of local anesthetics (i.e., memantine) for postoperative pain control or surgery may be worth practicing in the future.

Cocaine as the first local anesthetic was identified in 1860 [33]. Despite those somewhat physical or chemical differences, local anesthetics both induce cardiovascular and central nervous system toxicity [33]. For instance, bupivacaine causes significant cardiovascular toxicity clinically [34, 35]. It has been known that continuous intravenous injection of memantine appeared to lead to the later onset of cardiovascular and central nervous system toxicity than bupivacaine [5]. Moreover, axonal degeneration at the white matter and the spinal root was observed in rats receiving $>$ or $\leq$7.5% lidocaine [36]. Although it is not a replacement for lidocaine, there may be a potential for the use of memantine as a long-action local anesthetic in the clinical setting.

There are limitations to the study. We did not assess whether memantine caused neurotoxicity, however, it is noteworthy that in the neurobehavioral experiments we detected no apparent side effects after intrathecal injections. Histologic studies must
be examined in the future before the possible application of memantine as spinal analgesic in humans.

In summary, the preclinical data demonstrated that intrathecal memantine elicited a dose-dependent local anesthetic effect in spinal anesthesia. Memantine was less potent but greater duration of spinal blockades than lidocaine. Moreover, memantine, but not lidocaine, produced a markedly nociceptive-specific blockade. The neural block by memantine is potentially worth studying in the further.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support provided by the grant (MOST 103-2314-B-039 -004) from the Ministry of Science and Technology of Taiwan.

CONFLICTS OF INTEREST

No author has any conflict of interest related to the content of this paper.
REFERENCES


6 Chen Y.W., Tzeng J.I., Pan H.J., Hung C.H., Chen Y.C., Wang J.J. Co-administration of memantine with epinephrine produces a marked peripheral...


This article is protected by copyright. All rights reserved.


This article is protected by copyright. All rights reserved.
Memantine, a promising drug for the prevention of neuropathic pain in rat. Eur. J. 

21 Park J.W., Suh G.I., Shin H.E., Park G.E. Influence of memantine on nociceptive 
responses of the trigeminocervical complex after formalin injection. Cephalalgia: 
an international journal of headache (2012) **32** 308-316.

22 Olivan-Blazquez B., Herrera-Mercadal P., Puebla-Guedea M., Perez-Yus M.C., 
Andres E., Fayed N. et al. Efficacy of memantine in the treatment of fibromyalgia: 
A double-blind, randomised, controlled trial with 6-month follow-up. Pain (2014) 
**155** 2517-2525.

23 Fayed N., Olivan-Blazquez B., Herrera-Mercadal P., Puebla-Guedea M., 
Perez-Yus M.C., Andres E. et al. Changes in metabolites after treatment with 
memantine in fibromyalgia. A double-blind randomized controlled trial with 
magnetic resonance spectroscopy with a 6-month follow-up. CNS Neurosci. Ther. 
(2014) **20** 999-1007.

of post-mastectomy neuropathic pain with memantine: study protocol for a 
randomized controlled trial. Trials (2014) **15** 331.

25 Brock J.A., McLachlan E.M., Belmonte C. Tetrodotoxin-resistant impulses in 

This article is protected by copyright. All rights reserved.
11-17.

26 Novakovic S.D., Tzoumaka E., McGivern J.G., Haraguchi M., Sangameswaran L.,
Gogas K.R., et al. Distribution of the tetrodotoxin-resistant sodium channel PN3
18 2174-2187.

27 Hung C.H., Wang J.J., Chen Y.C., Chu C.C., Chen Y.W. Intrathecal
oxybuprocaine and proxymetacaine produced potent and long-lasting spinal

Comparison of local, epidural, and general anesthesia. N. Y. State J. Med. (1979)
79 1730-1733.

29 Lemoine S., Rouet R., Manrique A., Hanouz J.L. Effect of long-chain triglyceride lipid emulsion
on bupivacaine-induced changes in electrophysiological parameters of rabbit Purkinje cells.

30 Chen Y.W., Chu C.C., Chen Y.C., Leung Y.M., Wang J.J. Intrathecal chlorprothixene,
cis(z)-flupenthixol, chlorpromazine and fluphenazine for prolonged spinal blockades of sensory

31 Goldblum E., Atchabahian A. The use of 2-chloroprocaine for spinal anaesthesia.

This article is protected by copyright. All rights reserved.


34 Albright G.A. Cardiac arrest following regional anesthesia with etidocaine or bupivacaine. Anesthesiology (1979) 51 285-287.


Table 1. The 25% effective dose (ED$_{25}$), ED$_{50}$, and ED$_{75}$

<table>
<thead>
<tr>
<th></th>
<th>Motor function</th>
<th>Proprioception</th>
<th>Nociception</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED$_{50}$ (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memantine</td>
<td>2.01 (1.90–2.14)</td>
<td>1.81 (1.63–2.00)</td>
<td>1.69 (1.51–1.87)</td>
<td>1.30</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>1.02 (0.94–1.11)</td>
<td>0.97 (0.87–1.09)</td>
<td>0.90 (0.81–1.00)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

The ED$_{25}$, ED$_{50}$, and ED$_{75}$ of memantine and lidocaine (µmol) were obtained from Fig. 1. CI = confidence interval. Memantine, but not lidocaine, showed a more sensory-selective action over motor blockade ($P < 0.05$). The potencies of drugs in motor function, proprioception, and nociception (ED$_{50}$) were lidocaine greater than memantine ($P < 0.05$, for each comparison) by using 1-way ANOVA followed by pairwise Tukey’s HSD test.

Table 2. The percent of maximal possible effect (%MPE), duration, area under the curves (AUCs)

<table>
<thead>
<tr>
<th>%MPE</th>
<th>Duration (min)</th>
<th>AUCs (%MPE x min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete blockade time</td>
<td>Full recovery time</td>
</tr>
<tr>
<td>Motor function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memantine</td>
<td>100 ± 0</td>
<td>17.6 ± 4.3</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>100 ± 0</td>
<td>10.0 ± 1.4</td>
</tr>
<tr>
<td>Proprioception</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memantine</td>
<td>100 ± 0</td>
<td>21.0 ± 5.3</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>100 ± 0</td>
<td>10.0 ± 1.4</td>
</tr>
<tr>
<td>Nociception</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memantine</td>
<td>100 ± 0</td>
<td>30.1 ± 6.1</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>100 ± 0</td>
<td>24.1 ± 2.2</td>
</tr>
</tbody>
</table>

Spinal anesthesia (means ± S.E.M.) with memantine at 5 µmol and lidocaine at 3.25 µmol (n = 8 in each group). Of note, all of the animals in the memantine and lidocaine groups displayed complete blockade (100% MPE) of any function tested. Symbols (a, b, c) indicate $P < 0.05$, $P < 0.01$, $P < 0.001$ when memantine compared to lidocaine; Symbols (d, e) indicate $P < 0.01$, $P < 0.001$ when nociception compared to motor function.

This article is protected by copyright. All rights reserved.
Figure 1.

This article is protected by copyright. All rights reserved.
Figure 3.
FIGURE LEGENDS

**Fig. 1.** The dose—response curves of memantine and lidocaine on spinal blockades of motor function, proprioception, and nociception (% MPE) in rats (n = 8 at each testing point). Data are expressed as means ± S.E.M. %MPE = percent of maximal possible effect.

**Fig. 2.** Time courses of memantine (5 μmol) and lidocaine (3.25 μmol) on spinal blockades of motor function, proprioception, and nociception in rats. Values are presented as means ± S.E.M. Each testing point of the time course study contained eight rats.

**Fig. 3.** Duration (full recovery time) of action of memantine and lidocaine on spinal blockades of motor function, proprioception, and nociception at equipotent doses [50% effective dose (ED₅₀), ED₂₅, and ED₇₅] in rats (n = 8 at each testing point). Values are expressed as means ± S.E.M.