Neuroscience Letters 521 (2012) 115–118

Contents lists available at SciVerse ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Promazine and chlorpromazine for prolonged spinal anesthesia in rats

Yu-Wen Chen[a,b,*], Chin-Chen Chu[c,d], Yu-Chung Chen[e], Chung-Dann Kan[f], Jhi-Joung Wang[b]

[a] Department of Physical Therapy, China Medical University, Taichung, Taiwan
[b] Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan
[c] Department of Anesthesiology, Chi-Mei Medical Center, Tainan, Taiwan
[d] Department of Recreation and Health-Care Management, Chia Nan University of Pharmacy and Science, Tainan, Taiwan
[e] Division of Physical Therapy, Department of Physical Medicine and Rehabilitation, Cheng Hsin General Hospital, Taipei, Taiwan
[f] Department of Surgery, National Cheng Kung University Medical Center, Tainan, Taiwan

HIGHLIGHTS

- Intrathecal promazine and chlorpromazine displayed spinal anesthesia.
- Promazine and chlorpromazine were less potent than bupivacaine on spinal anesthesia.
- Promazine and chlorpromazine produced longer spinal block duration than bupivacaine.

ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 21 May 2012
Accepted 25 May 2012

Keywords:
Chlorpromazine
Promazine
Bupivacaine
Spinal blockade
Motor function
Nociception

ABSTRACT

Though promazine and chlorpromazine elicited cutaneous anesthesia, no study of spinal anesthesia with chlorpromazine and promazine has been reported. This study was to examine whether chlorpromazine and promazine produce spinal anesthesia. Using a rat model *via* intrathecal injection, we tested spinal blockades of motor function and nociception by promazine, chlorpromazine or bupivacaine, and so were dose–response studies and durations. We demonstrated that chlorpromazine and promazine elicited dose-dependent spinal blockades in motor function and nociception. On the 50% effective dose (ED_{50}) basis, the rank of potency of these drugs was bupivacaine > promazine > chlorpromazine ($P < 0.05$ for the differences). On an equipotent basis (25% effective dose [ED_{25}], ED_{50}, and ED_{75}), the block duration caused by chlorpromazine or promazine was longer than that caused by the long-lasting local anesthetic bupivacaine ($P < 0.01$ for the differences). Chlorpromazine and promazine, as well as bupivacaine, showed longer duration of sensory block than that of motor block. Our data reported that intrathecal promazine and chlorpromazine with a more sensory-selective action over motor blockade had less potent and longer-lasting spinal blockades when compared with bupivacaine.

The typical antipsychotics began with the serendipitous discovery of the antipsychotic activity of chlorpromazine, one of phenothiazine-type antipsychotics including a phenothiazine ring with different substituents attached at the 2 and 10 positions [12]. Besides, there is a growing body of evidence that chlorpromazine blocks the voltage-gated Na^+ currents [17,18]. One of the most extensive pharmacological studies of chlorpromazine was demonstrated that chlorpromazine (1%) produced anesthesia of the sciatic nerve of guinea pigs [5], and it had been recently known that chlorpromazine and promazine elicited infiltrative anesthesia of skin in rats [8].

Long-lasting local anesthetics are frequently administered intrathecally for various procedures or pathologies [3], and spinal anesthesia is a relatively simple technique, which gives adequate surgical conditions *via* injecting a small amount of local anesthetic [11]. To the best of our knowledge, no study of spinal anesthesia with chlorpromazine and promazine has been reported to date. The aim of this study was to examine, using a rat model of spinal punctures, whether chlorpromazine and promazine produced long-acting spinal anesthesia and then compared with bupivacaine, a long-lasting local anesthetic.

The experimental protocols were approved by the Institutional Animal Care and Use Committee of China Medical University (Taiwan), and conformed to the recommendations and policies of the International Association for the Study of Pain (IASP). Male Sprague-Dawley rats (295–345 g) were purchased from the National Laboratory Animal Center, Taipei, Taiwan. They were housed in groups of three, with food and water freely available

* Corresponding author at: Department of Physical Therapy, China Medical University, No. 91 Hsueh-Shih Road, Taichung 40402, Taiwan.
Tel.: +886 4 22053366x7327; fax: +886 4 22065051.
E-mail address: cywhwok@mail.cmu.edu.tw (Y.-W. Chen).

0304-3940/$ – see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.neulet.2012.05.067

until the time of experiments. The climate controlled room maintained at 24 °C with approximately 50% relative humidity on a 12 h light/dark cycle (6:00 AM–6:00 PM).

Promazine HCl, chlorpromazine HCl, and bupivacaine HCl were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). All drugs were freshly prepared in 5% dextrose as solution before intrathecal injections. After intrathecal injections, the low pH of these plain solutions (ranging from 5.3 to 7.1) is likely to be buffered quickly by the cerebral spinal fluid (pH 7.4).

Two studies were carried out. In study 1, in a dose-dependent manner, the potencies of promazine (0.38, 0.54, 0.60, 0.75, 1.00, 1.35 and 1.75 μmol), chlorpromazine (0.50, 0.63, 0.75, 1.00, 1.50, 1.75 and 2.50 μmol), and bupivacaine (0.18, 0.23, 0.32, 0.43, 0.75 and 0.90 μmol) on spinal anesthesia were performed (n = 8 rats for each dose of each drug). Then, the spinal anesthetic effects of promazine at 1.75 μmol and chlorpromazine at 2.50 μmol were compared with those of bupivacaine at 0.90 μmol (n = 8 rats for each dose of each drug). In study 2, on an equipotent basis (25% effective dose [ED_{25}], ED_{50}, and ED_{75}), the spinal block duration (full recovery time) caused by promazine or chlorpromazine was compared with that caused by bupivacaine (n = 8 rats for each dose of each drug).

All animals were handled to familiarize them with the experiments and to minimize stress-induced analgesia before intrathecal injections. The drugs were injected intrathecally on unanaesthetized rats as previously described [7,13]. In brief, a 27-gauge needle attached to a 50 μl syringe (Hamilton, Reno, Nevada) was inserted into the midline of the lumbar 4–5 (L4–L5) intervertebral space and 50 μl of drugs was injected. Rats were then checked for paralysis of two hind limbs, indicative of a spinal blockade. Rats that displayed unilateral blockades were excluded from the study and killed by using an overdose of sevoflurane.

For consistency, one experienced investigator, who was blinded to the identity of the injected drugs, was responsible for handling all the animals and behavioral evaluation. Rats were evaluated before medication and at 1, 3, 5, 7, 10, 15, and 20 min afterwards, then again at 10 min interval until 1 h, at 15 min interval until 2 h, and at 30 min interval until 6 h. The magnitude of spinal blockades was described as the percent of possible effect (PE%). The maximum blockade in a time course of spinal anesthesia with drugs was described as the percent of maximal possible effect (MPE%).

After intrathecal injections, nociception and motor function were evaluated as previously described [6,10]. In brief, nociception was evaluated according to the withdrawal reflex or vocalization elicited by pinching a skin fold on each rat's back at 1 cm from the proximal part of the tail, the lateral metatarsus of the two hind limbs, and the dorsal part of the mid-tail. Nociceptive blockade was graded as 0 (absent or 100% MPE), 1 (75% MPE), 2 (50% MPE), 3 (25% MPE), and 4 (normal nociception or 0% MPE) [9]. At each testing time, only one pinch was given to each of the four testing areas, and the time interval between stimulations at different areas was around 2 s.

Motor function was assessed by measuring 'the extensor postural thrust' of the right hind limb of each rat and was measured as the gram force, which resisted contacting the platform *via* the heel applied to the digital platform balance (Mettler Toledo, PB 1502-S, Switzerland). The decrease in force, resulting from extensor muscle tone, was considered motor deficit (block). The pre-injection control value was considered a 0% motor blockade or 0% MPE, and a force less than 20 g was interpreted as a 100% motor blockade or 100% MPE [6].

After intrathecally injecting the rats with 6–7 different doses of each drug (n = 8 for each dose of each drug), dose–response curves were constructed. The curves were then fitted by using SAS NLIN procedures (SAS Institute Inc., Carey, NC), and the value of ED_{50}, defined as the doses that caused 50% spinal blockades of motor function and nociception, were obtained [10,15]. The ED_{25} and ED_{75} of drugs were obtained *via* using the same SAS NLIN procedures that were used to derive the ED_{50}. On an equipotent basis (ED_{25}, ED_{50}, and ED_{75}), the full recovery time (duration) of each blockade, defined as the interval from drug injection to full recovery (0% MPE), was measured and compared. Furthermore, that area under curves (AUCs) of spinal blockades of drugs was estimated *via* using Kinetica version 2.0.1 (InnaPhase Corporation, Philadelphia, PA).

Values are presented as mean ± SEM or ED_{50} values with 95% confidence interval (95% CI). The differences in the ED_{50}s (Table 1) among drugs or the differences in the MPE%, duration, and AUCs of drugs (Table 2) were evaluated by one-way analysis of variance (ANOVA), followed by the pairwise Tukey's honest significance difference (HSD) test. The differences in durations (Fig. 3) among drugs were evaluated by two-way ANOVA followed by pairwise Tukey's HSD test. SPSS for Windows (version 17.0) was used for all statistical analyses. Statistical significance was set at $P < 0.05$.

Chlorpromazine and promazine, as well as bupivacaine, showed a dose-dependent effect on spinal anesthesia in rats (Fig. 1). The

Table 1
The 50% effective dose (ED_{50}), ED_{25}, and ED_{75} of drugs with 95% confidence interval (95% CI) on spinal blockades of motor function and nociception in the rat.

Drug	Motor function	Nociception	Mean		
	ED_{50} (95% CI)	ED_{50} (95% CI)	ED_{25}	ED_{50}	ED_{75}
Bupivacaine	0.34 (0.32–0.36)	0.28 (0.26–0.30)	0.22	0.31	0.87
Promazine	0.78 (0.74–0.84)	0.70 (0.68–0.73)	0.56	0.75	0.98
Chlorpromazine	1.14 (1.07–1.23)	0.97 (0.90–1.05)	0.74	1.06	1.51

EDs of drugs (μmol) were obtained from Fig. 1. The potency ranking (ED_{50}) of spinal anesthesia with these tested drugs was bupivacaine > promazine > chlorpromazine ($P < 0.05$ for the differences) using one-way ANOVA and then the pairwise Tukey's HSD test.

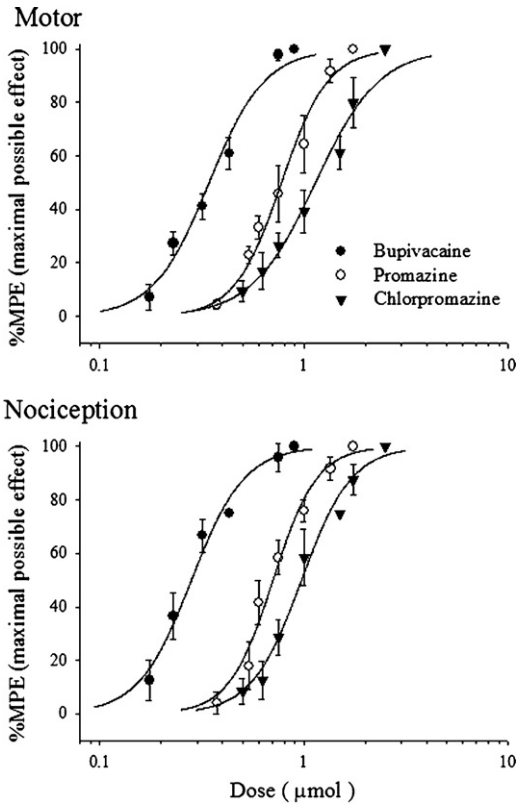

Fig. 1. The dose–response curves of promazine, chlorpromazine, and bupivacaine on spinal blockades (% MPE) of motor function and nociception in rats (n = 8 at each testing point). Data are mean ± SEM. % MPE = percent of maximal possible effect.