Doxepin is a tricyclic antidepressant (TCA) and has been widely used in the treatment of major depression and other psychiatric disorders [1]. Recently, doxepin and other TCAs have also been found to be effective in the treatment of various acute and chronic painful conditions, including postoperative pain, low back pain, and neuropathic pain [2–6]. Doxepin, like other TCAs, exerts its action through several mechanisms, such as blockade of α2-adrenergic, N-methyl-D-aspartate, and histaminergic H2 receptors, and inhibition of the reuptake of serotonin and norepinephrine [2–7]. Doxepin has also recently been found to be effective in blocking the use-dependent and voltage-gated Na+ channels [8,9]. This phenomenon of Na+ channel blockade was also found with local anesthetics in clinical use [10].

Following perisciatic nerve injection, doxepin further produced a significant neural blockade on the sciatic nerve [9,11]. Following intrathecal injection, doxepin also produced a significant spinal anesthetic effect in a single-dose study [12]. These important results suggested that doxepin has a local anesthetic effect. However, although several workers suggested that doxepin has a local anesthetic effect, studies related to its spinal action have been relatively inconclusive. First, no dose-response study was carried out to evaluate the spinal action of doxepin. Second, no duration study was carried out to evaluate the duration of its action. Third, no comparisons were made between doxepin and the traditional local anesthetics on their potencies and duration of action. The aim of our study was to evaluate and compare the potency and duration of the spinal anesthetic effect of doxepin with two commonly used traditional local anesthetics, bupivacaine and lidocaine.
MATERIALS AND METHODS

Male Sprague-Dawley rats, obtained from the National Laboratory Animal Center, Taiwan (weight between 300 and 350 g), were used. They were housed in groups of three for at least 1 week in a climate-controlled room maintained at 21°C with approximately 50% relative humidity. Lighting was on a 12-hour light/dark cycle (lights on at 6:00 a.m.), with food and water available on demand except during the time of testing. Each treatment group (that is, for each drug of each dose) consisted of six rats. All tests were performed in accordance with the recommendations and policies of the International Association for the Study of Pain, and the protocol was approved by the animal investigation committee of Chi-Mei Medical Center.

In part 1 of the study, the potencies of doxepin, bupivacaine, and lidocaine were evaluated for their spinal anesthetic effect. Part 2 evaluated the durations of their spinal anesthetic effect.

Doxepin HCl, bupivacaine HCl, and lidocaine HCl were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in 5% dextrose as a solution. Rats were handled before testing to familiarize them with the experiment and to minimize stress-induced analgesia. The experimenter was blinded to the drugs and dosages used. Intrathecal injections of drugs were performed according to the method reported previously [12]. In brief, the injections of drugs were performed in conscious rats following adequate local anesthesia by infiltration with 1.0% lidocaine (50 µL) around the injection site of lumbar intervertebral space 4 to 5 (L4–5). Following local infiltrative anesthesia, a 27-gauge needle attached to a 100-µL syringe (Hamilton, Reno, NV, USA) was inserted intrathecally through the midline of the L4–5 intervertebral space and 90 µL of drug was instilled. Success in intrathecal injection was confirmed by a sense of ‘give’ and the sign of a tail flick [12,13]. Each rat received only one intrathecal injection of drug.

In part 1, the potencies of the drugs’ action were evaluated. Following intrathecal injections of drugs (n = 6 rats for each drug of each dose), three neurobehavioral examinations that evaluated motor function, proprioception, and nociception were conducted according to the method reported previously [11,12]. Briefly, (1) motor function was evaluated by measuring the strength of extensor postural thrust of the hindlimbs of rats, (2) proprioception was evaluated by measuring the functional deficit of a hopping response following waving the animal body while it was standing on just one hindlimb, and (3) nociception was evaluated by measuring the withdrawal reflex or vocalization elicited by pinches of the skinfold over the back 1 cm from the proximal part of the tail, the lateral metatarsus of the bilateral hindlimbs, and the dorsal part of the mid-tail. Rats were tested at 5 minutes before medication, at 1, 5, and 10 minutes afterward, again at 10-minute intervals until 1 hour had passed, and at 15-minute intervals until 2 hours had passed. For consistency, one trained examiner was responsible for all rat handling and behavioral evaluations. The magnitude of spinal blockade (motor function, proprioception, and nociception) was described as the percentage of possible effect (% PE) [12]; the maximum value of % PE values following each test was presented as percentage of maximum possible effect (% MPE). Dose–response curves were constructed following tests, and the 50% effective doses (ED50 values) of the drugs were then obtained from computer-derived curve fitting by SAS NLIN analysis (SAS Institute Inc., Cary, NC, USA) of the dose–response curves. The ED50 was defined as the dose of a drug that caused a 50% spinal block of motor function, proprioception, or nociception following intrathecal administration [14].

In part 2, the durations of the drugs’ action were evaluated. The ED25 and ED75 values of drugs were obtained from computer-derived curve fitting as described in part 1. Rats then received intrathecal injections of drug at doses of ED25, ED50, or ED75 (n = 6 rats for each drug of each dose). However, because there were three ED50 values for each drug (that is, ED50 values for motor function, proprioception, and nociception), the mean value of these three ED50 values was used for injection. The mean values of ED25 and ED75 were also obtained by the above method. The duration of the spinal anesthetic effect of drug was defined as the full recovery time of spinal blockade. It was measured as an interval from time zero at the time of injection to the time of complete recovery [11,12].

The differences of ED50 values among drugs were evaluated by using a one-way analysis of variance (ANOVA) followed by the pairwise Tukey HSD test. The differences of duration among drugs were evaluated by a two-way ANOVA followed by the pairwise Tukey HSD test. Statistical significance was defined as p < 0.05.

RESULTS

The effects in rat spinal blockade in motor function, proprioception, and nociception of doxepin and two traditional local anesthetics at various doses were evaluated. Because of the similarities of the values, only those obtained
from doxepin and bupivacaine at an administered dose of 0.45 \( \mu \text{mol} \) are shown (Figure 1). At this dose, doxepin provided 81.1%, 84.8%, and 75.0% of blockades (% MPE) in motor function, proprioception, and nociception, respectively, whereas bupivacaine provided 74.2%, 75.2%, and 70.8% of blockades, respectively.

The dose-response curves were constructed following intrathecal injections of drugs of different doses (Figure 2). The ED\textsubscript{50} values of drugs for the spinal blockades of motor function, proprioception, and nociception are given in the Table. Doxepin, bupivacaine, and lidocaine produced dose-related spinal blockades of motor function, proprioception, and nociception. The potency of spinal blockade by doxepin was similar to that of bupivacaine and higher than that of lidocaine (Table). All rats recovered completely after intrathecal injections of drugs. The durations of spinal blockades of drugs are shown in Figure 3. Among the drugs evaluated, doxepin had the longest duration of action (\( p < 0.001 \), in motor function, proprioception, and nociception).

**DISCUSSION**

In this study, the potencies and durations of the spinal anesthetic effects of doxepin and two traditional local anesthetics were evaluated and compared. We found that doxepin produced dose-related spinal anesthetic effects of motor function, proprioception, and nociception that were more potent than those of lidocaine and longer lasting than those of bupivacaine and lidocaine.

Doxepin, a TCA, has been used in the treatment of major depression and other psychiatric disorders for longer than 40 years [1]. Recently, doxepin and other TCAs have also been used in the treatment of pain [2–6,15,16]. In animal studies, doxepin dose-dependently increased the mechanical pain threshold of the paw in rats [6]. Doxepin given 30 minutes before intraplantar formalin also significantly increased the inflammatory pain threshold of the paw in rats [6]. In human studies, doxepin administered preoperatively significantly decreased postoperative opioid requirements [6]. Doxepin also effectively relieved lower back pain and neuropathic pain in patients [15,16]. Although doxepin was effective in alleviating pain, the detailed mechanism of its action is not clear [2]. The proposed mechanism was that doxepin, a TCA, might enhance the inhibitory descending cortical, supraspinal, and spinal pathways that might mitigate nociceptive impulses from the peripheral to the central nervous system [2–6]. This mechanism might involve the inhibition of the reuptake of serotonin and norepinephrine, and the blockade of the muscarinic, cholinergic, histaminergic, \( \alpha \)-adrenergic, N-methyl-D-aspartate receptors [2–6]. Recently, doxepin and several other TCAs were also found to have a blocking effect on Na\(^+\) conductance by a manner similar to the clinically used local anesthetics [10,12,14]. However, although doxepin appeared to have a local anesthetic effect, studies related to the spinal anesthetic effect of doxepin were relatively inconclusive. Here, we demonstrate that doxepin, acting as a local anesthetic, dose-dependently blocked the spinal functions of motor activity, proprioception, and nociception. In comparison with bupivacaine, a potent and long-acting traditional local anesthetic, doxepin produced an equipotent local anesthetic effect but with longer duration of action. This result was valuable, not only pharmacologically, but also clinically. If doxepin is appropriately used, its specific characteristics may further enhance its clinical value in the management of pain. The spinal actions of doxepin may also give some explanations of its clinical effect in pain management.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Motor function ED\textsubscript{50} (95% CI)</th>
<th>Proprioception ED\textsubscript{50} (95% CI)</th>
<th>Nociception ED\textsubscript{50} (95% CI)</th>
<th>Mean ED\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doxepin</td>
<td>0.32 (0.30–0.35)*</td>
<td>0.30 (0.27–0.33)*</td>
<td>0.32 (0.29–0.36)*</td>
<td>0.31</td>
</tr>
<tr>
<td>Bupivacaine</td>
<td>0.36 (0.34–0.38)</td>
<td>0.35 (0.33–0.37)</td>
<td>0.35 (0.33–0.37)</td>
<td>0.35</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>1.33 (1.27–1.38)</td>
<td>1.29 (1.21–1.38)</td>
<td>1.19 (1.12–1.26)</td>
<td>1.27</td>
</tr>
</tbody>
</table>

CI = confidence interval.

ED\textsubscript{50} values (\( \mu \text{mol} \)) of drugs tested were obtained from computer-derived curve fitting by SAS NLIN analysis of the dose-response curves shown in Figure 2. *\( p < 0.001 \) when compared with lidocaine, by using a one-way ANOVA followed by the pairwise Tukey HSD test. The difference between doxepin and bupivacaine was not significant.
Figure 1. The effects of spinal blockade (percentage of possible effect, % PE) of doxepin and bupivacaine (mean ± SE) in rats ($n = 6$ in each group). The injected dose was 0.45 mmol. Neurologic evaluation was obtained before, and at 1, 5, 10, 20, 30, 40, 50, 60, 75, and 90 minutes after drug injection.

Figure 2. The dose-response curves of spinal blockade of doxepin (D), bupivacaine (B), and lidocaine (L) following intrathecal injections in rats ($n = 6$ for each drug of each dose). Data (mean ± SE) were fitted by SAS NLIN analysis.
CONCLUSIONS

Intrathecal doxepin produced dose-related spinal anesthetic effects of motor function, proprioception and nociception that were more potent than that of lidocaine and longer than that of bupivacaine and lidocaine. The spinal actions of doxepin may provide some explanation of its clinical effect in pain management.

REFERENCES

在大鼠 Doxepin 具有強效與長效之脊髓麻醉效用
鄭伯智 陳柄仁 陳郁文 朱光興 程廣義 王志中 諸錦承
奇美醫學中心 外科部暨麻醉部
2高雄醫學大學附設中和紀念醫院 麻醉學科

Doxepin 是一個三環抗憂鬱藥物，最近被發現具有治療多種急性與慢性疼痛之效用。然而，它的作用機轉特別是在脊髓作用尚不清楚。本研究的目的在評估 Doxepin 之脊髓麻醉效用。本研究以兩個傳統且臨床常用的局部麻醉劑 Bupivacaine 及 Lidocaine 為對照組，在雄性 Sprague-Dawley 大鼠評估藥物的作用強度與持續作用時間。我們發現如同 Bupivacaine 和 Lidocaine 一樣，Doxepin 也具有和劑量相關之阻斷運動、本體感覺及疼痛之脊髓麻醉效用。在藥物間，Doxepin 產生比 Lidocaine 還強的脊髓麻醉效用 (p < 0.001，在每一個比較中) 且比 Bupivacaine 和 Lidocaine 還長效 (p < 0.001，在每一個比較中)。本實驗證實在大鼠脊髓腔內注射 Doxepin 可產生和劑量相關之阻斷運動、本體感覺與疼痛之脊髓麻醉效用，此效用比 Lidocaine 還強且比 Bupivacaine 和 Lidocaine 還長。Doxepin 之脊髓作用也許可以部份解釋其臨床止痛效用。

關鍵詞：麻醉，脊髓腔內，大鼠，脊髓的，三環抗憂鬱藥
(高雄醫誌 2006;22:68—74 )

收文日期：94 年 9 月 28 日
接受刊載：94 年 11 月 2 日
通訊作者：褚錦承醫師
台南奇美醫學中心麻醉部
台南縣永康市中華路 901 號