The Systemic Toxicity of Equipotent Proxymetacaine, Oxybuprocaine, and Bupivacaine During Continuous Intravenous Infusion in Rats

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BACKGROUND: Although proxymetacaine and oxybuprocaine produce topical ocular and spinal anesthesia, they have never been tested as cutaneous anesthetics. We compared cutaneous analgesia of proxymetacaine and oxybuprocaine with bupivacaine and tested their central nervous system and cardiovascular toxicity.
METHODS: After blockade of cutaneous trunci muscle reflex with subcutaneous injections, we evaluated the local anesthetic effect of proxymetacaine and oxybuprocaine on cutaneous analgesia in rats. After IV infusions of equipotent doses of oxybuprocaine, proxymetacaine, and bupivacaine, we observed the onset time of seizure, apnea, and impending death and monitored mean arterial blood pressure and heart rate.
RESULTS: Proxymetacaine and oxybuprocaine acted like bupivacaine and produced dose-related cutaneous analgesia. On a 50% effective dose basis, the ranks of potencies were proxymetacaine > oxybuprocaine > bupivacaine ($P < 0.01$). Under equipotent doses, the infusion times of proxymetacaine or oxybuprocaine required to cause seizure, apnea, and impending death were longer than that of bupivacaine ($P < 0.05$). The decrease in mean arterial blood pressure and heart rate was slower with oxybuprocaine and proxymetacaine compared with bupivacaine ($P < 0.05$ for the differences) at equipotent doses.
CONCLUSIONS: Oxybuprocaine and proxymetacaine were more potent at producing cutaneous anesthesia but were less potent than bupivacaine at producing central nervous system and cardiovascular toxicity.
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Topical ocular anesthesia has been part of ophthalmology for more than a century.[1] The most frequently used drugs include proparacaine (proxymetacaine), benoxinate (oxybuprocaine), tetracaine, lidocaine, and bupivacaine. Oxybuprocaine and proxymetacaine (Fig. 1), 2 ester anesthetics, are frequently used drugs for topical ocular anesthesia because of the ease of administration and minimal side effects.[1] Clinically, ocular anesthesia is performed with topical 0.5% proxymetacaine for patients undergoing strabismus surgery and posterior vitrectomy[2,3] and with topical 0.4% oxybuprocaine for penetrating trabeculectomy, repair of a ruptured globe, and cataract surgery.[4–6] Many publications have reported the successful treatment of trigeminal neuralgia by topical anesthetic oxybuprocaine or proxymetacaine instilled in the eye of the affected side.[4,5]

Injection of local anesthetics into tissues is a recommended method for postoperative pain control and surgical anesthesia because it produces relatively few side effects.[7] However, the technique is limited by the short duration of analgesia or anesthesia.[8] For this reason, bupivacaine is chosen for infiltration because of its longer duration of effective analgesia.[9] Recently, we showed that oxybuprocaine and proxymetacaine were more potent at producing spinal anesthesia in rats, when compared with bupivacaine or lidocaine.[10] Oxybuprocaine and bupivacaine produced similar durations of spinal blockade and a more sensory-selective action over motor blockade.[10] However, cutaneous analgesia after infiltration of oxybuprocaine and proxymetacaine has not been evaluated. In this study, we compared infiltration anesthesia of oxybuprocaine and proxymetacaine with bupivacaine. We also evaluated the systemic toxicity of the 3 drugs by infusing equipotent doses of the drugs.

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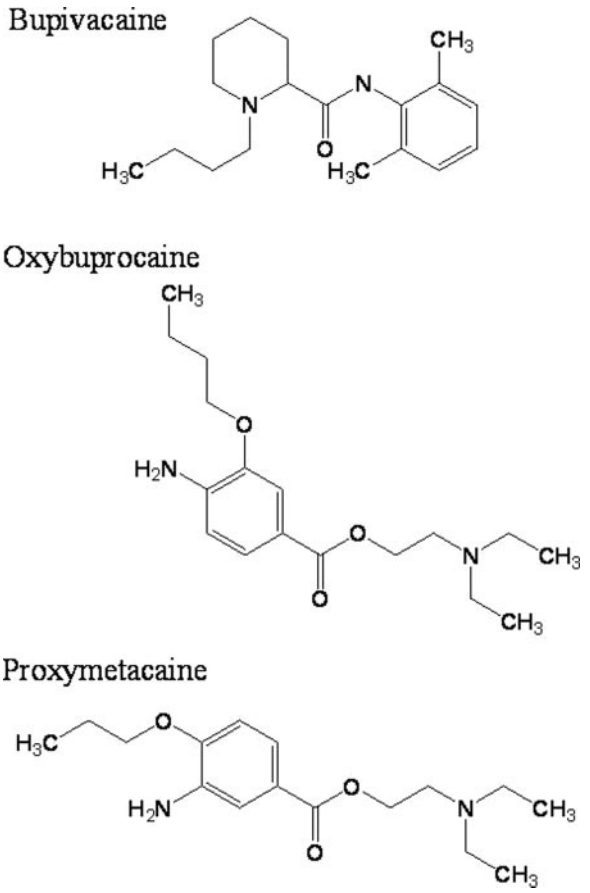

Figure 1. The chemical structures of bupivacaine, oxybuprocaine, and proxymetacaine.

METHODS

Animals

Male Sprague-Dawley rats weighing 275–325 g were obtained from the National Laboratory Animal Centre, Taipei, Taiwan. Sixty-four rats were housed in groups of 3, with food and water freely available until the time of testing. The climate-controlled room was maintained at 24°C with approximately 50% humidity and a 12-h light/dark cycle (6:00 AM to 6:00 PM). The experimental protocols were approved by the Animal Investigation Committee of China Medical University, Taichung, Taiwan and conformed to the recommendations and policies of the International Association for the Study of Pain.

Drugs

Proxymetacaine HCl (proparacaine HCl), oxybuprocaine HCl (benoxinate HCl), and bupivacaine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). All drugs were freshly prepared in normal saline (0.9% NaCl) before the subcutaneous injections or IV infusion.

Infiltrative Cutaneous Analgesia

Before subcutaneous injections, the hair on the rats' dorsal surface of the thoracolumbar region (10 × 10 cm^2) was mechanically removed. Each drug was injected into a naïve area of the rat's shaved back. The back was divided into 4 quadrants (to clearly demarcate injection and control sites), and each rat received each study drug. Each animal was injected twice with the drug being tested and separated by an interval not <5 days. Subcutaneous injections of drugs were performed as reported previously.[11] Briefly, 0.6 mL of the drugs was injected subcutaneously at the dorsal surface of the thoracolumbar region of the unanesthetized rats. After subcutaneous injection, the wheal was marked with ink within 30 s after injection. A von Frey (No. 15, Somedic Sales AB, Stockholm, Sweden) filament (19 g), to which the cut end of an 18-gauge needle was affixed, was used to standardize the stimulus intensity on the rat's skin. Six pinpricks (at 6 different points within each wheal) with a frequency of 0.5–1 Hz were used in each testing. Each drug's cutaneous anesthetic effect was evaluated quantitatively as the number of times the pinprick failed to elicit a response of cutaneous trunci muscle reflex. For example, the complete absence of 6 responses was defined as complete block (100% of possible effect [PE]). During the test, the maximum value of %PE was presented as percent of maximal PE (%MPE). Each drug's duration of action was defined as the time from drug injection (i.e., time = 0) to full recovery of cutaneous trunci muscle reflex (no analgesic effect was found or 0% of MPE recorded).[11]

After subcutaneously injecting the rats with different doses of each drug (n = 8 for each dose of each drug), dose-response curves were constructed using the %MPE for each dose of each drug. The curves were then fitted using a computer-derived SAS NLIN analysis (SAS Institute, Carey, NC, version 9.1; SPSS for Windows, version 12.0), and the values of 50% effective dose (ED_{50}), defined as the doses that caused 50% cutaneous analgesia, were obtained.[12,13] Drug potencies were compared with dose responses. Durations of drug effect defined as the intervals from injection to complete recovery were measured.

Measurements of Systemic Toxicity and Hemodynamic Variables

Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (45 mg/kg), and the right femoral artery and vein were cannulated with polyethylene catheters (PE-50), which were filled with heparin saline (30 U/mL). The free end of the catheter was threaded through an 18-gauge needle and then tunneled subcutaneously. The catheter was cut with 5 cm protruding from the skin at the midline in the posterior cervical area and sealed by heating it with a match and compressing it with a hemostat.[14]

On Day 2, the animals were placed in a small cage with an open top to allow the lines to reach the animal and prevent the animal from chewing on the lines. The tube in the femoral artery was connected to a transducer, and mean arterial blood pressure (MAP) and

Table 1. Baseline Data are Showed as Mean ± SEM

Variable	Saline	Bupivacaine	Proxymetacaine	Oxybuprocaine
Body weight	302 ± 18	298 ± 8	311 ± 10	307 ± 10
MAP	107 ± 3	105 ± 3	103 ± 3	105 ± 1
HR	419 ± 12	400 ± 13	438 ± 15	413 ± 19

There were no significant differences among the groups for these variables.

MAP = mean arterial blood pressure; HR = heart rate.

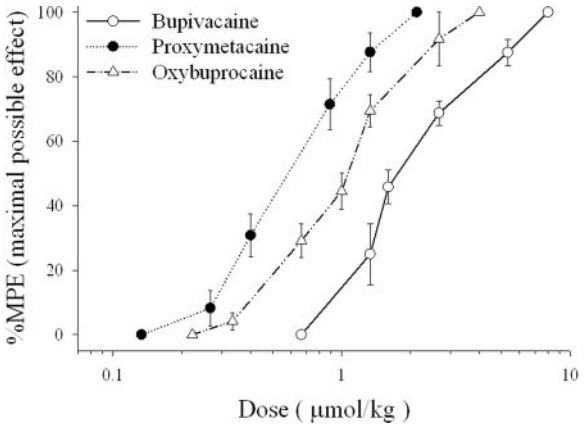

Figure 2. The dose-response curves of proxymetacaine, oxybuprocaine, and bupivacaine on infiltrative cutaneous analgesia in rats ($n = 8$ at each testing point). Data are presented as mean ± SEM.

Table 2. The 50% Effective Doses (ED_{50}s) of Drugs

Drug	ED_{50}s (95% CI)
Bupivacaine	1.89 (1.68–2.12)
Proxymetacaine	0.59 (0.52–0.68)
Oxybuprocaine	1.01 (0.93–1.09)

ED_{50}s of drugs (μmol/kg) were obtained from Figure 2.

Potencies of drugs (ED_{50}s) were proxymetacaine > oxybuprocaine > bupivacaine ($P < 0.01$, for each comparison) using a 1-way analysis of variance followed by pairwise Tukey's honestly significant difference test.

CI = confidence interval.

heart rate (HR) were recorded using a polygraph (MP36, BIOPAC Systems, Goleta, CA). The tube in the right femoral vein was connected to an infusion pump (Harvard Model 22 Infusion Pump, Harvard Apparatus, Holliston, MA) for delivery of the drugs.[15] The investigator (C-HH) was blinded to the drugs under study. After IV infusions of either 1) bupivacaine at 8.00 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, proxymetacaine at 3.56 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, or oxybuprocaine at 6.67 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ or 2) normal saline ($n = 7$) at a rate of 400 $\mu L \cdot kg^{-1} \cdot min^{-1}$, the onset time of seizure, respiratory arrest, time to cause impending death, MAP, and HR were evaluated. Rats were evaluated 2 min before infusion medication and at 5-min intervals to 105 min.

The onset time of seizure was defined as the time when the first convulsion occurred[14] and respiratory arrest when apnea occurred for 15 s by observation of chest movement. The time to impending death was defined as the time it took for the HR to decrease to 0 per minute.

Statistical Analysis

Values are presented as mean ± SEM or ED_{50} values with 95% confidence interval. The differences in ED_{50} values among drugs were evaluated by a 1-way analysis of variance (ANOVA) followed by pairwise Tukey's honestly significant difference test. The differences in baseline data and the time to cause toxicity between medications were evaluated using 1-way ANOVA and then the pairwise Tukey's honestly significant difference test. ANOVA with repeated measures followed by Duncan's multiple-range test was used for *post hoc* multiple comparisons of means on MAP and HR. SPSS for Windows (version 12.0) was used for all statistical analyses. Statistical significance was set at $P < 0.05$.

RESULTS

The baseline data of body weight, MAP, and HR showed no significant differences among groups (Table 1). Proxymetacaine, oxybuprocaine, and bupivacaine produced dose-dependent infiltration anesthesia in rats (Fig. 2). The ED_{50} values of drugs are shown in Table 2. For ED_{50}, the relative potency of these drugs was found to be proxymetacaine > oxybuprocaine > bupivacaine (Table 2). All rats recovered completely after each subcutaneous injection. At dosages of 8.00 μmol/kg for bupivacaine, 3.56 μmol/kg for proxymetacaine, and 6.67 μmol/kg for oxybuprocaine, all the local anesthetic drugs caused 100% blockade with durations of actions of 110 ± 11, 93 ± 9, and 128 ± 8 min, respectively (Figs. 2 and 3). Saline produced no infiltration anesthetic effects.

At equipotent doses, the times required to cause seizure, respiratory arrest, and impending death were longer in the oxybuprocaine group than in the bupivacaine or proxymetacaine group (Fig. 4). MAP and HR showed a tendency to decrease before cardiovascular (CV) collapse (Fig. 5) in all study groups. The decreases in MAP and HR were slower in the proxymetacaine or oxybuprocaine groups compared with the bupivacaine group (Fig. 5). The rapidity of decrease of MAP and HR occurred in the following order: bupivacaine > proxymetacaine > oxybuprocaine (Fig. 5).

DISCUSSION

Our study showed that proxymetacaine, oxybuprocaine, and bupivacaine produced dose-dependent infiltration anesthesia. At equipotent doses, systemic toxicity after IV proxymetacaine or oxybuprocaine occurred later compared with bupivacaine.

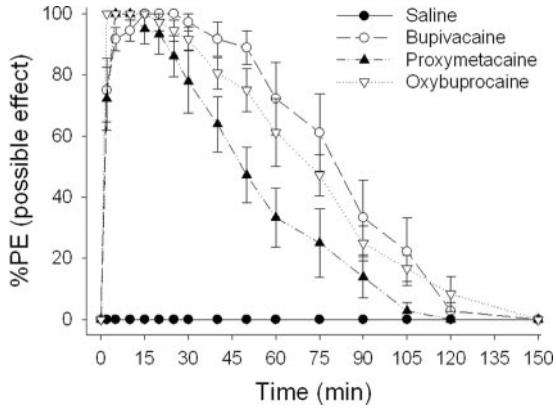

Figure 3. Time courses of infiltrative cutaneous analgesia of proxymetacaine at 3.56 μmol/kg, oxybuprocaine at 6.67 μmol/kg, and bupivacaine at 8.00 μmol/kg in rats. Data are presented as mean ± SEM; each group, $n = 8$.

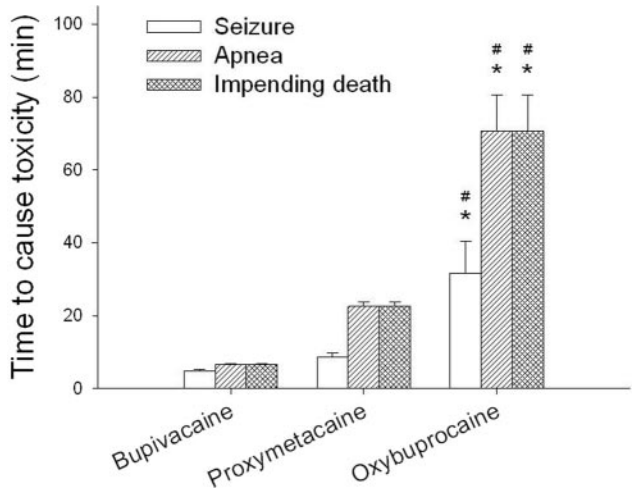

Figure 4. Time to cause toxicity (at equipotent doses) of bupivacaine, proxymetacaine, and oxybuprocaine at the onset of seizure, respiratory arrest, and time to cause impending death. Toxicity symptoms were not detected in the saline group ($n = 7$) (data not shown). Symbols (*) indicate $P < 0.05$ for proxymetacaine or oxybuprocaine compared with bupivacaine. Data are presented as mean ± SEM.

Infiltrative cutaneous anesthesia is an attractive option for management of postoperative pain and surgical anesthesia because it is relatively free of side effects.[16] In this study, proxymetacaine and oxybuprocaine were found to have a local anesthetic effect that was more potent than bupivacaine, a long-acting local anesthetic. Recently, we showed that intrathecal injections of oxybuprocaine, proxymetacaine, bupivacaine, and lidocaine produced dose-related spinal anesthesia.[10] Proxymetacaine and oxybuprocaine produced almost 4.1- and 2.4-fold greater potency, respectively, than did bupivacaine, when used as a spinal anesthetic in rats.[10] Based on dose-response curves, proxymetacaine and oxybuprocaine were more potent than bupivacaine.[10] There seems to be a uniformity of the comparative potencies of proxymetacaine, oxybuprocaine, and bupivacaine with respect to cutaneous analgesia and spinal anesthesia.

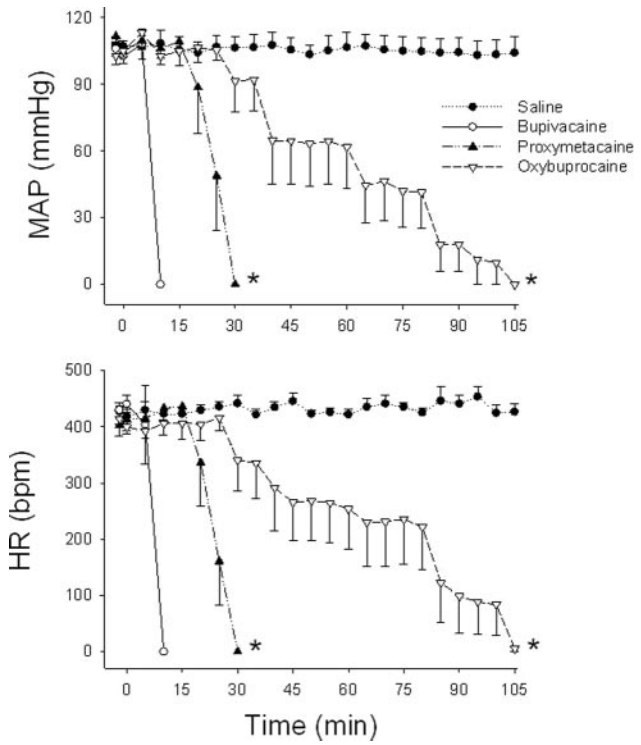

Figure 5. Mean arterial blood pressure (MAP) and heart rate (HR) change during infusion of either 1) bupivacaine ($n = 8$) at 8.00 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, proxymetacaine ($n = 8$) at 3.56 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, or oxybuprocaine ($n = 8$) at 6.67 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ or 2) normal saline ($n = 7$) in the volume of 400 $\mu L \cdot kg^{-1} \cdot min^{-1}$ (the same volume given to the animals in the drug group) as infusion; 0 min is the start of infusion. Infusion was stopped when the time to cause impending death was reached. Symbols (*) indicate $P < 0.05$ for proxymetacaine or oxybuprocaine compared with bupivacaine. Data are presented as mean ± SEM.

Accidental IV injection of local anesthetic may induce central nervous system and CV system toxicity and even cause death.[14] In this study, oxybuprocaine was less potent at producing toxicity compared with bupivacaine or proxymetacaine. However, the degrees of toxicities were the same once toxicity occurred (Fig. 4). We also noted that the decreases in MAP and HR were longer with oxybuprocaine and proxymetacaine compared with bupivacaine. Overall, these results suggest that oxybuprocaine and proxymetacaine are less toxic and better tolerated than bupivacaine after IV injection.

Our study showed that local anesthetic potency does not necessarily mean increased CV toxicity in that proxymetacaine and oxybuprocaine were more potent local anesthetics yet caused less CV toxicity than bupivacaine. It is possible that the 2 drugs are unique local anesthetics. Also, in the previous CV toxicity studies,[17] dogs' lungs were ventilated to maintain their acid-base status and Po_2 at normal levels. However, the role of acidosis and hypoxia is not clear because these occur rapidly after the onset of local anesthetic-induced convulsions in humans.[18] The different results may also have been attributable to species

differences or in the experimental methods. Our results need to be confirmed by other investigators.

In conclusion, our study showed that oxybuprocaine and proxymetacaine are more potent at producing infiltration anesthesia compared with bupivacaine. Intravenous equipotent doses of proxymetacaine or oxybuprocaine produce lower central nervous system and CV system toxicity than bupivacaine. There seems to be a greater margin of safety between the anesthetic dose and the dose that produces toxicity for proxymetacaine or oxybuprocaine compared with bupivacaine. The clinical relevance of these effects warrants further investigation.

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