

# 中國醫藥大學

## 專題研究計畫成果報告

計畫名稱：右美沙芬與右啡烷結合 clonidine 應用於  
大鼠浸潤性皮膚止痛效果評估

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Title: Clonidine as adjuvant for oxybuprocaine or dextrorphan has a significant peripheral action in intensifying and prolonging the local anaesthetic effect on infiltrative cutaneous analgesia

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**Abstract: Purpose** The aim of the study was to evaluate co-administration of clonidine with oxybuprocaine or dextrorphan on infiltrative cutaneous analgesia and to see whether it could have a peripheral action in enhancing duration of local anaesthesia.

**Methods** Cutaneous analgesia was evaluated by a block of the cutaneous trunci muscle reflex, which is characterized by reflex movement of the skin over the back produced by twitches of lateral thoracispinal muscles in response to local dorsal cutaneous noxious pinprick. The analgesic effect of the addition of clonidine with oxybuprocaine and dextrorphan for subcutaneous injection was evaluated. Oxybuprocaine, a common used local anaesthetic, was used as control.

**Results** On an ED50 basis, the rank of drug potency was oxybuprocaine > dextrorphan. Mixtures of clonidine with oxybuprocaine or dextrorphan (ED50 or ED95) extended the duration of drug action and increased the potency of cutaneous analgesia. Clonidine at the dose of 0.12 µmol did not produce cutaneous analgesia.

**Conclusions** Oxybuprocaine showed more potent cutaneous analgesia than dextrorphan. Co-administration of oxybuprocaine or dextrorphan with clonidine increased the potency and duration on cutaneous analgesia in rats.

**Key Words:** clonidine, oxybuprocaine, dextrorphan, cutaneous analgesia

1 Clonidine as adjuvant for oxybuprocaine or dextrorphan has a  
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4 significant peripheral action in intensifying and prolonging the  
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7 local anaesthetic effect on infiltrative cutaneous analgesia  
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48 Short heading (40 characters or less): clonidine enhances dextrorphan and  
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50 oxybuprocaine  
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54 Summary: Clonidine enhances and prolongs dextrorphan and oxybuprocaine  
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57 cutaneous analgesia.  
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## Abstract

**Purpose** The aim of the study was to evaluate co-administration of clonidine with oxybuprocaine or dextrorphan on infiltrative cutaneous analgesia and to see whether it could have a peripheral action in enhancing duration of local anaesthesia.

**Methods** Cutaneous analgesia was evaluated by a block of the cutaneous trunci muscle reflex, which is characterized by reflex movement of the skin over the back produced by twitches of lateral thoracispinal muscles in response to local dorsal cutaneous noxious pinprick. The analgesic effect of the addition of clonidine with oxybuprocaine and dextrorphan for subcutaneous injection was evaluated.

Oxybuprocaine, a common used local anaesthetic, was used as control.

**Results** On an ED<sub>50</sub> basis, the rank of drug potency was oxybuprocaine > dextrorphan. Mixtures of clonidine with oxybuprocaine or dextrorphan (ED<sub>50</sub> or ED<sub>95</sub>) extended the duration of drug action and increased the potency of cutaneous analgesia. Clonidine at the dose of 0.12 µmol did not produce cutaneous analgesia.

**Conclusions** Oxybuprocaine showed more potent cutaneous analgesia than dextrorphan. Co-administration of oxybuprocaine or dextrorphan with clonidine increased the potency and duration on cutaneous analgesia in rats.

**Key Words:** clonidine, oxybuprocaine, dextrorphan, cutaneous analgesia

## Introduction

Oxybuprocaine (benoxinate) produces the dose-related cutaneous analgesia<sup>1</sup> and spinal anaesthesia<sup>2</sup> in rats. At equipotent doses, systemic toxicity following intravenous oxybuprocaine and proxymetacaine occurred later compared to bupivacaine.<sup>1</sup> Dextrorphan is a sodium channel blocker<sup>3</sup> which produces dose-related local anaesthetic effects on cutaneous analgesia,<sup>4,5</sup> spinal or sciatic nerve blockades in motor function, proprioception and nociception in rats.<sup>6,7</sup> Besides, dextrorphan was similar to bupivacaine and displayed a long-acting cutaneous analgesia.<sup>5</sup> Previous studies from our laboratory extend those studies by showing the addition of epinephrine with dextrorphan administered directly to the subcutaneous region produces the additive cutaneous analgesia.<sup>5</sup>

The addition of clonidine, an  $\alpha_2$ -adrenoreceptor agonist, to low concentration of ropivacaine or bupivacaine can extend the duration of sensory block and analgesic time in children.<sup>8</sup> Furthermore, clonidine added to mepivacaine selectively enhanced sensory blockade after midhumeral block,<sup>9</sup> and co-administration of clonidine prolonged local anaesthetic effect of lidocaine on the forearm of volunteer subjects.<sup>10</sup> The benefits of adding clonidine to local anaesthetics are decreasing postoperative analgesic requirement and improving analgesic quality.<sup>11-13</sup>

Co-injection of clonidine and lidocaine to each forearm of volunteer subjects has

1 a significant peripheral action in enhancing duration of local anaesthesia.<sup>10</sup> The aim of  
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4 the study was to evaluate co-administration of clonidine with oxybuprocaine or  
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7 dextrorphan on cutaneous analgesia and to see whether it could have a peripheral  
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10 action in enhancing duration of local anaesthesia after a single subcutaneous injection.  
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13 We suggested that the impact of adding clonidine to subcutaneous injections of  
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16 oxybuprocaine or dextrorphan could enhance and prolong the local anaesthetic effects  
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20 on cutaneous analgesia in rats.  
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## Methods

### Animals

Male Sprague-Dawley rats weighting 200-250 g were obtained from the National Laboratory Animal Centre (Taipei, Taiwan) and then housed in a climate controlled room, with food and water available *ad libitum* up to time of testing. The climate-controlled room was maintained at 22°C with approximately 50% relative humidity on a 12-h light/dark cycle (6:00 AM–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

Benoxinate (oxybuprocaine) HCl, dextrorphan tartrate and clonidine HCl were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in 0.9% NaCl (saline).

### Experimental Procedures

Three experiments were carried out. In experiment 1, time courses of oxybuprocaine (1.20, 0.60, 0.15, 0.03  $\mu\text{mol}$ ), dextrorphan (9.00, 6.00, 3.00, 0.60  $\mu\text{mol}$ ), and clonidine (0.96, 0.48, 0.24, 0.12  $\mu\text{mol}$ ) on cutaneous analgesia were evaluated ( $n = 8$  rats for each dose of each drug). In experiment 2, the %MPE,



1 duration, and area under curves (AUCs) of oxybuprocaine and dextrorphan (ED<sub>50</sub> or  
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4 ED<sub>95</sub>) with/without clonidine were assessed on cutaneous analgesia (n = 8 rats for  
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7 each dose of each drug). In experiment 3, after the above testings, one control group  
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10 was further added into the study to rule out the possibility of systemic effect of drugs  
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13 on cutaneous analgesia. Groups (n = 8 rats for each dose of each drug) received  
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16 subcutaneous injection (the right calf of the rats) of testing drug (oxybuprocaine or  
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19 dextrorphan) with a dose of 2ED<sub>95</sub> or clonidine with a dose of 0.96 µmol.  
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### 22 23 **Subcutaneous Injection of Drug** 24

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26 All rats were handled daily up to 7 days to minimize the stress on the rats during  
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28 experiments and generally improve their experimental performance before  
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30 experiments.<sup>1,5</sup> On the day before subcutaneous injections, the hair on the rats' dorsal  
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33 surface of the thoracolumbar region (10×6 cm<sup>2</sup>) was mechanically shaved. The  
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36 subcutaneous injection of drug was performed as reported previously.<sup>1,5</sup> In brief, the  
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39 drugs in 0.6 mL were injected subcutaneously using a 30-gauge needle in  
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42 unanesthetized rats at the dorsal surface of the thoracolumbar region. After  
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45 subcutaneous injection, a circular elevation of the skin, a wheal, approximately 2 cm  
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48 in diameter occurred. The wheal was marked with ink within one minute after  
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51 injection. For consistency, one experienced investigator (Dr. Chen Y.W.) who was  
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54 blinded to the drugs injected was responsible for assessing the cutaneous anaesthetic  
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1 effect. The drugs were prepared and injected by another investigator (Li Y.T.).  
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#### 4 **Neurobehavioral Evaluation**

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7 Cutaneous anaesthesia was evaluated by the cutaneous trunci muscle reflex  
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9 (CTMR), characterized by the reflex movement of the skin over the back produced by  
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11 twitches of the lateral thoracospinal muscle in response to local dorsal cutaneous  
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13 stimulation after drug injections.<sup>1,5</sup> A Von Frey filament (No.15; Somedic Sales AB,  
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15 Stockholm, Sweden), to which the cut end of an 18-gauge needle was affixed, was  
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17 used to produce the standardized nociceptive stimulus ( $19\pm 1$  g). After observing an  
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19 animal normal reaction to pinpricks applied outside the wheal and on the contralateral  
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21 side, we applied six pinpricks (at six different points with a frequency of 1-2 Hz)  
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23 inside the wheal and scored the number to which the rat failed to react. Cutaneous  
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25 analgesic effect of each drug was assessed quantitatively as the number of times the  
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27 pinprick failed to elicit a response, with, for example, the complete absence of six  
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29 responses was defined as complete nociceptive block (100% of possible effect; 100%  
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31 PE). The test of six pinpricks was applied 5 min before drug injection, at 0, 2 and 5  
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33 min afterwards, then again every 5 min after injection for the first 30 min, every 10  
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35 min after injection for 30-60 min, and every 15-30 min thereafter until the CTMR  
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37 fully recovered from the block. During the test, the maximum blockade in a time  
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39 course of cutaneous anaesthesia of drugs was described as the percent of maximum  
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1 possible effect (%MPE). The duration of action of each drugs was defined as the time  
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4 from drug injection (i.e., time=0) to full recovery of CTMR (no analgesic effect was  
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7 found or 0% MPE recorded).<sup>1,5</sup>  
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### 10 **Evaluation of 50% Effective Dose (ED<sub>50</sub>) and ED<sub>95</sub>**

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13 After subcutaneously injecting the rats with four doses of each drug ( $n = 8$  for  
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16 each dose of each drug), dose-response curves were obtained via the % MPE for each  
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19 dose of each drug. The value of 50% or 95% effective dose (ED<sub>50</sub> or ED<sub>95</sub>), defined as  
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22 the dose that caused 50% or 95% cutaneous analgesia, was obtained via a  
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25 computer-derived SAS NLIN analysis (SPSS for Windows, version 17.0; SAS  
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28 Institute Inc., Carey, NC).<sup>14</sup>  
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### 32 **Statistical Analysis**

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35 Data are presented as mean  $\pm$  SD (range) or ED<sub>50</sub> value with 95% confidence  
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38 interval (95% CI). Data were evaluated by the Student's t-test. A statistical software,  
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41 SPSS for Windows (version 17.0, SPSS, Inc, Chicago, IL, USA), was used, and a  $P$   
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44 value less than 0.05 was considered statistically significant.  
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## Results

### The Potency of Drugs on Cutaneous Analgesia

The structures of oxybuprocaine and dextrorphan are shown in Figure 1. Subcutaneous injections of oxybuprocaine and dextrorphan produced dose-dependent cutaneous analgesia in rats (Fig. 2). Clonidine alone at the doses of 0.12-0.96  $\mu\text{mol}$  showed no cutaneous analgesia (data not shown). The  $\text{ED}_{50}$ s and  $\text{ED}_{95}$ s of drugs constructed from Figure 2 were demonstrated in Table 1. On an  $\text{ED}_{50}$  basis, the rank of drug potency was oxybuprocaine > dextrorphan (Table 1).

### Cutaneous Analgesia of Drugs at $\text{ED}_{95}$ with/without Clonidine

At the dose of  $\text{ED}_{95}$ , oxybuprocaine for 92% sensory blockade with AUC of  $2394 \pm 782$  and dextrorphan for 88% sensory blockade with AUC of  $2515 \pm 551$  are displayed in Figure 2 and Table 2. Subcutaneous injection of clonidine alone at the dose of 0.12  $\mu\text{mol}$  demonstrated no cutaneous anaesthesia (Fig. 3). After drugs at the dose of  $\text{ED}_{95}$  were co-injected with clonidine (0.12  $\mu\text{mol}$ ), oxybuprocaine and dextrorphan caused 100% sensory blockade (100% MPE) with AUCs of  $10812 \pm 1727$  and  $8360 \pm 1108$ , respectively (Fig. 3 and Table 2). The %MPE, duration and AUCs of drugs at the dose of  $\text{ED}_{95}$  with clonidine were greater ( $P < 0.05$ ) than drugs without clonidine in Table 2.

### Cutaneous Analgesia of Drugs at $\text{ED}_{50}$ with/without Clonidine

1           When drugs at the dose of ED<sub>50</sub> were co-injected with clonidine (0.12 μmol),  
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4 complete sensory blockade (100% MPE) in the oxybuprocaine (8 of 8 rats) group  
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7 occurred, but not in the dextrorphan (5 of 8 rats) group. Compared with drugs at the  
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10 dose of ED<sub>50</sub> alone, drugs (ED<sub>50</sub>) co-injected with 0.12 μmol clonidine, the AUCs  
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13 were increased in oxybuprocaine group from 699 ± 142 to 7401 ± 561 (*P*<0.001 and  
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16 in dextrorphan group from 916 ± 361 to 4821 ± 590 (*P*<0.001), respectively.  
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### 19 **One Control Group**

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23           Neither the calf subcutaneous injection of clonidine (0.96 μmol) nor calf  
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26 subcutaneous injections of oxybuprocaine and dextrorphan (2ED<sub>95</sub>) demonstrated  
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29 cutaneous analgesia on the back of the rat, loss of motor activity or sedation (data not  
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32 shown). All rats recovered completely after each subcutaneous injection of drug.  
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## Conclusions

In this report we showed for the first time that oxybuprocaine was a more potent local anaesthetic on cutaneous analgesia than dextrorphan. Clonidine dramatically enhances and prolongs the sensory blocking effect of oxybuprocaine and dextrorphan on cutaneous analgesia in rats. Subcutaneous injection of clonidine (0.96-0.12  $\mu\text{mol}$ ) produces no cutaneous analgesia.

The clinical relevance of this prolonged sensory blockade, though useful in certain situations, may be limited overall, when considering the higher incidence of motor blockade caused by clonidine.<sup>15</sup> Both of motor blockade following central neuraxial block and also of sedative effects of clonidine should be limited. Given these dose-limiting central side effects, clonidine may be beneficial to apply peripherally. Therefore, this study evaluated the cutaneous analgesic effect of local anaesthetics combined with clonidine. In this study, we found that co-administration of clonidine with oxybuprocaine or dextrorphan enhanced the duration and prolonged the cutaneous analgesia in rats. Because we have already known that clonidine prolongs the local anaesthetic duration of lidocaine and bupivacaine,<sup>10,16</sup> it is also not surprising that clonidine prolongs the cutaneous analgesic duration of oxybuprocaine and dextrorphan, two local anaesthetics.<sup>1, 2, 4, 5</sup>

A single subcutaneous injection of clonidine (1.2 mg/kg) resulted in delayed

1 tactile hypersensitivity 24–34 h after clonidine administration in rats.<sup>17</sup> Clonidine  
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4 hydrochloride (0.1 mg/kg, i.p.) did not affect the tactile allodynia and mechanical  
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7 hyperalgesia induced from orthotropic inoculation with melanoma into the hind paw  
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10 of mice.<sup>18</sup> These experiments used higher doses of clonidine. Typically, these were  
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13 selected from published articles as the highest doses that were used to study  
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16 antinociception or drug tolerance.<sup>17,18</sup> Our study displayed that the low dose of  
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19 clonidine (0.12-0.96  $\mu\text{mol}$ ; 0.14-1.14 mg/kg), an  $\alpha_2$ -adrenoreceptor agonist, did not  
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22 produce cutaneous analgesia. However, both scientific and clinical studies have also  
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25 provided evidence for the mechanism of action of clonidine as a local anaesthetic  
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28 additive, as well as suggesting local anaesthetic-like properties of clonidine itself.<sup>19,20</sup>  
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32 It may be explained that  $\alpha_1$ -adrenoceptor agonists (e.g. epinephrine and  
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35 phenylephrine) at low doses between 3.5  $\mu\text{mol}$  – 1.0  $\mu\text{mol}$  can mainly act by mixed  
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38 subtypes of  $\alpha_1$ -adrenoceptor to induce the local anaesthetic activity.<sup>21</sup>  
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42 To rule out the possibility of systemic analgesic effect of drugs (oxybuprocaine,  
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45 dextrorphan, and clonidine), one control group was used. subcutaneous injection (the  
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48 right calf of the rats) of testing drug We demonstrated subcutaneous injections of a  
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51 large dose of drugs into the right calf of the rats did not produce cutaneous analgesia.  
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55 In addition, after observing an animal normal reaction to pinpricks applied outside the  
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58 wheal and on the contralateral side, we applied six pinpricks inside the wheal and  
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1 scored the number to which the rat failed to react. These results support our finding  
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4 that the cutaneous analgesic effect of clonidine with oxybuprocaine and dextrorphan  
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7 were due to their local action on the skin. Our study agrees with those results, which  
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10 showed that clonidine (0.01 mg) has significant peripheral action in enhancing local  
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13 anaesthesia duration on subcutaneous co-infiltration with lidocaine.<sup>10</sup>  
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17 In this study, duration (AUC) of drugs (oxybuprocaine and dextrorphan) at the  
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19 dose of ED<sub>95</sub> with clonidine was approximately 4.5- and 3.3-folds greater than drugs  
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21 without clonidine, respectively. We also evaluated the local anaesthetic at the dose of  
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23 ED<sub>50</sub> with/without clonidine. Results demonstrated that clonidine as an adjuvant for  
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26 oxybuprocaine and dextrorphan increased the potency of the local anaesthetic effect  
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29 on cutaneous analgesia. Adding clonidine rather than increasing doses of drugs may  
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32 be an option to increase the cutaneous analgesic effect of drugs. In addition, duration  
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35 (AUC) of drugs (oxybuprocaine and dextrorphan) at the dose of ED<sub>50</sub> with clonidine  
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38 was almost 10.6- and 5.3-folds greater than drugs without clonidine, respectively.  
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42 Coadministration of oxybuprocaine with clonidine extended the longer duration on  
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45 infiltrative cutaneous analgesia than dextrorphan. Mixtures of local anaesthetics (e.g.  
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48 oxybuprocaine or dextrorphan) and clonidine might be practiced on infiltration  
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51 anaesthesia of skin incision sites for the surgery and postoperative pain relief, but this  
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54 should be explored in future studies.  
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1 Cutaneous anaesthesia using local anaesthetics is an acceptable option for  
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4 surgical anaesthesia and management of postoperative pain because it is relatively  
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7 free of adverse effects.<sup>22</sup> Oxybuprocaine and dextrorphan had a local anaesthetic  
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10 effect on cutaneous analgesia.<sup>1,5</sup> This study demonstrated that oxybuprocaine showed  
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13 more potent cutaneous analgesia than dextrorphan. We also displayed that  
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16 oxybuprocaine or dextrorphan with clonidine prolonged the duration and enhanced  
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19 the potency of cutaneous analgesia. It has been mentioned that clonidine, clinically  
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22 added to preparations of local anaesthetics, prolonged the duration of action via three  
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25 possible mechanisms. First, clonidine may cause local vasoconstriction, thus  
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28 decreasing local anaesthetic spread and removal around nerves.<sup>23</sup> Secondly, clonidine  
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31 blocked C and A $\delta$  fibres as a consequence of an increase in K<sup>+</sup> conductance in  
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34 isolated neurons, thus intensifying local anaesthetic conduction block.<sup>19</sup> Thirdly,  
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37 spinal clonidine combined with local anaesthetics or used in peripheral nerve  
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40 blockades intensifies and prolongs anaesthesia.<sup>23,24</sup>  
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45 Dextrorphan with epinephrine produced an additive effect on infiltrative  
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48 cutaneous analgesia,<sup>5</sup> but the cutaneous analgesic effect of adding clonidine to  
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51 dextrorphan showed a synergistic effect (Fig. 3). The previous study indicated that  
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54 epinephrine mainly acts through mixed subtypes of  $\alpha$ 1-adrenoceptor to induce  
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57 cutaneous analgesic activity.<sup>21</sup> These data suggested that dextrorphan and  
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1  $\alpha$ 2-adrenoceptor might interact and contribute the cutaneous analgesia. The structure  
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4 of oxybuprocaine is similar to cocaine, the origin structure of most local anaesthetics,  
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7 and both of them contain an ester-linkage structure. However, the structure of  
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10 dextrorphan is different from that of oxybuprocaine in Figure 1. Coadministration of  
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13 oxubuprocaine, a topical anaesthetic, with clonidine produced a synergistic effect on  
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16 cutaneous analgesia. This result is in agreement on the benefits of adding clonidine to  
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20 dextrorphan and oxybuprocaine for infiltration.  
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23 We concluded that oxybuprocaine produced more potent cutaneous analgesia  
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26 than dextrorphan. Addition of clonidine to oxybuprocaine or dextrorphan has a  
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29 significant peripheral action in intensifying and prolonging the local anaesthetic effect  
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32 on cutaneous analgesia in rats.  
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**Table 1.** The 50% effective doses (ED<sub>50</sub>s) and ED<sub>95</sub>s of oxybuprocaine and dextrorphan on infiltrative cutaneous analgesia.

Drug	ED <sub>50</sub> s ( 95% CI )	ED <sub>95</sub> s
Oxybuprocaine	0.23 (0.19 – 0.28)	1.29
Dextrorphan	2.47 (2.03 – 3.00)	8.45

ED<sub>50</sub>s of drugs (  $\mu\text{mol}$  ) were obtained from Figure 2. CI = confidence interval. Potencies of drugs ( ED<sub>50</sub>s ) were oxybuprocaine > dextrorphan ( $P<0.01$ , for each comparison).



**Table 2.** The %MPE, duration and AUCs of oxybuprocaine and dextrorphan on infiltrative cutaneous analgesia.

	%MPE	Duration (min)		AUCs (%MPE×min)
		Complete blockade	Full Recovery	
OX ED <sub>50</sub>	50 ± 9	0	25 ± 2	713 ± 142
OX ED <sub>50</sub> + CL	100 ± 0***	38.5 ± 6.5***	104 ± 10***	7401 ± 561***
DX ED <sub>50</sub>	50 ± 13	0	37 ± 9	916 ± 361
DX ED <sub>50</sub> + CL	93 ± 9***	2.1 ± 2.7*	97 ± 8***	4821 ± 590***
OX ED <sub>95</sub>	92 ± 9	2.9 ± 3.9	50 ± 10	2494 ± 782
OX ED <sub>95</sub> + CL	100 ± 0**	70.5 ± 22.5***	155 ± 25***	10812 ± 1727***
DX ED <sub>95</sub>	88 ± 12	0.3 ± 0.5	57 ± 11	2515 ± 551
DX ED <sub>95</sub> + CL	100 ± 0**	44.8 ± 13.3***	127 ± 16***	8360 ± 1108***
CL	0	0	0	0

Percent of maximum possible effect (%MPE), duration of drug action, area under curves (AUCs) for cutaneous analgesia (mean ± SD) for oxybuprocaine or dextrorphan with/without clonidine (n = 8 in all groups). Symbols (\*, \*\*, \*\*\*) indicate  $P < 0.05$ ,  $P < 0.01$ ,  $P < 0.001$  when drug alone compared with drug in the presence of clonidine, respectively. OX, oxybuprocaine; DX, dextrorphan; CL, clonidine 0.12 µmol.

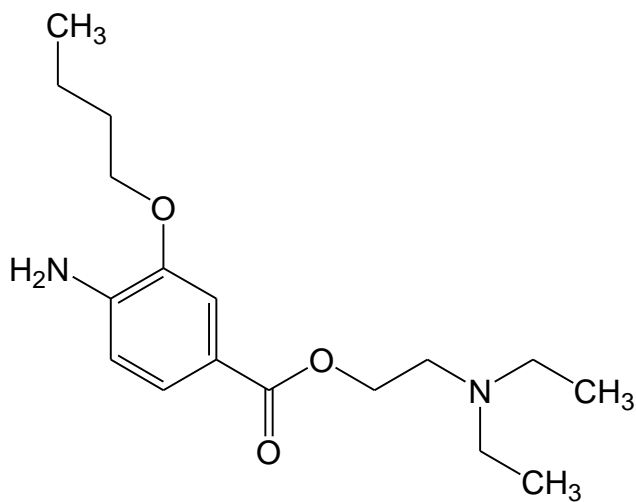
## Legends to figures

**Figure 1.** The chemical structure of oxybuprocaine and dextrorphan.

**Figure 2.** Time courses (four doses in each group) of oxybuprocaine and dextrorphan performed on infiltrative cutaneous analgesia in rats. Drugs were tested and results presented as dose-dependent curves, respectively. Values are expressed as mean  $\pm$  SD; n = 8 rats for each dose of each drug.

**Figure 3.** The addition of clonidine (CL) with oxybuprocaine or dextrorphan (ED<sub>50</sub> or ED<sub>95</sub>) and clonidine alone on infiltrative cutaneous analgesia in rats. Clonidine at the dose of 0.12  $\mu$ mol produces no cutaneous analgesia. Values are expressed as mean  $\pm$  SD; n = 8 rats for each dose of each drug. ED<sub>50</sub> or ED<sub>95</sub> means 50% or 95% effective dose, respectively.

Oxybuprocaine



Dextrorphan

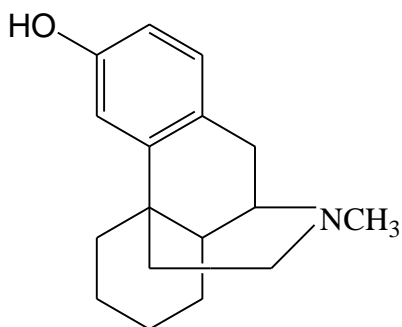
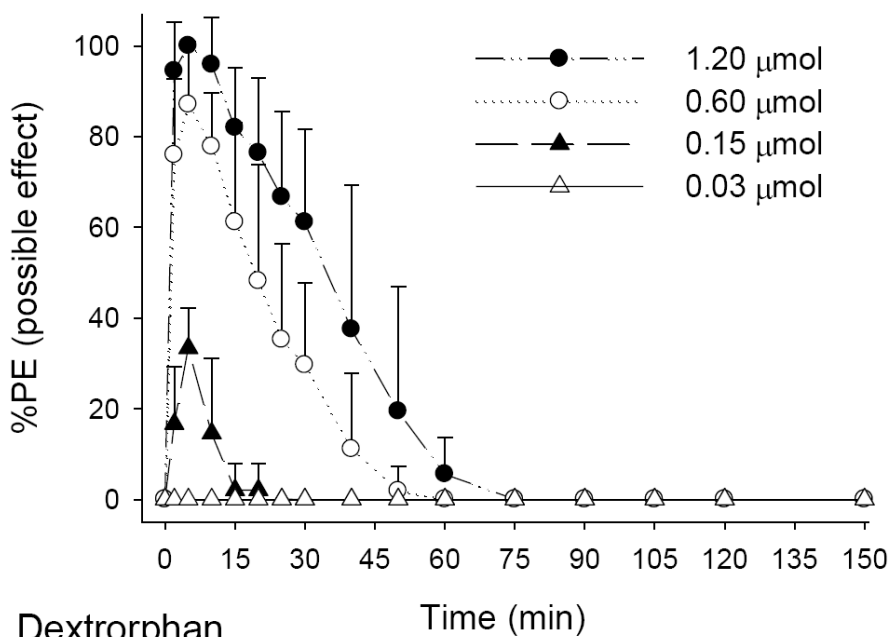


Fig. 1.

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## Oxybuprocaine



## Dextrorphan

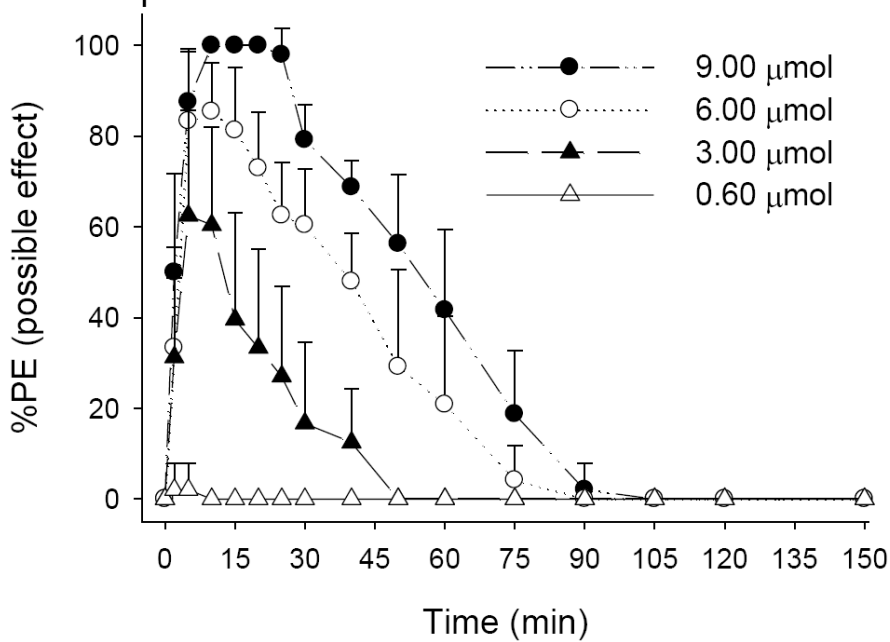


Fig. 2.

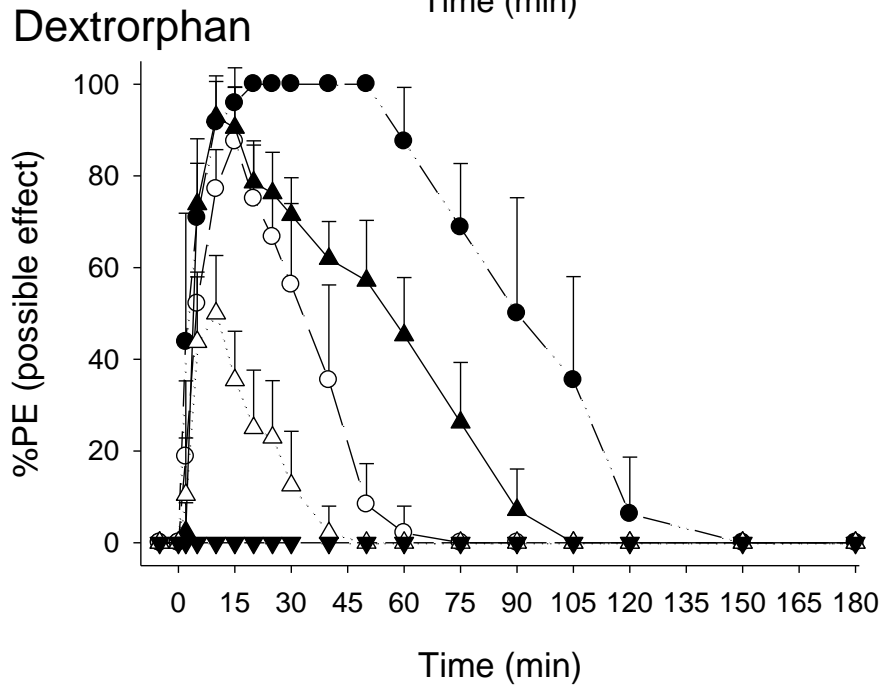
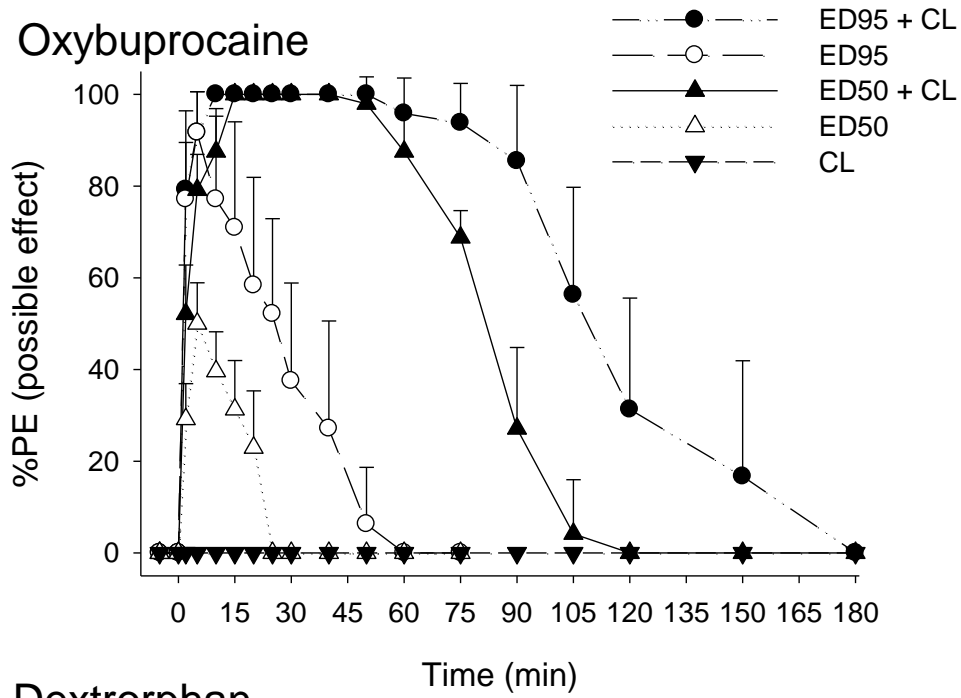


Fig. 3.

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