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Original article

Local infiltration of the surgical wound with levobupivacaine, ibuprofen, and epinephrine in postoperative pain: An experimental study

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ABSTRACT

The body areas from where sutures are removed later, where wound healing is delayed. Epidural analgesia is the most effective method but could not be used for postoperative pain. Peripheral nerve blockers also provided excellent analgesia but are not effective in postoperative pain. Infiltration of the surgical wound with local anesthetics is decreased postoperative pain by inhibiting transmission of noxious impulses at the site. The objective of the study was to explore the effect of the local infiltration of the surgical wounds with low-dose of levobupivacaine, ibuprofen, and epinephrine over the sutured muscle wound in postoperative pain. Laparotomy was performed in adult rats under isoflurane anesthesia. During surgery, the surgical wounds were infiltrated with 50 µL solution containing 0.3% w/v levobupivacaine, 2 mg/mL ibuprofen, and 8 mg/mL epinephrine (treatment group) and compared to infiltration of that of water for injection (vehicle group) over the sutured muscle wound before skin closing. Postoperative pain was assessed by rodent grimace scales scoring. The study also carried out for measurement for histopathological examinations and the tensile strength of wound. The one-way ANOVA following the Dunnett Multiple comparisons test was used to show significant differences between parameters at 95% level of confidence. The fall in pain started with three-hour post-surgery in the treatment group. At 24 h after the end of the successful infiltration, the treatment group had significant reduction of a pain than vehicle group ($p = 0.048$; $q = 3.527$). After three weeks of the wound were closed, a significant improvement of angiogenesis process ($p = 0.021$) and the tensile strength ($p = 0.019$) for the treatment group as compared to baseline. The experimental study was reported that local infiltration of the surgical wound with levobupivacaine, ibuprofen, and epinephrine combination was effective in the postoperative pain and healing of the surgical wounds.

1. Introduction

In the postoperative period, 80% of individuals suffer from pain with almost all describing pain as moderate to severe [1]. Postoperative pain may lead to increase post-surgical hospitalization stay, insomnia, discomfort [2]. In some cases, after surgery, patients are unable to take oral medications. Here intravenous non-steroidal anti-inflammatory drug (NSAID) or transdermal patches of opioids are used [3].

Epidural analgesia is the most effective method with fewer side effects [4]. However, it could not be used in routine practice for postoperative pain management because of having complications related to epidural and spinal blockade [5]. Peripheral nerve blockers also provided excellent analgesia but are not effective in postoperative pain management because of having limitation of the single dose application [6]. The present studies are reported that bupivacaine [7] and ropivacaine [8] have cardiac toxicity and chondrotoxicity [9], so must be used studiously. There is significant injection site pain with the intravenous administration, specifically when employing a rapid infusion [3]. The body areas from where sutures are removed later like back, lower extremity, and in tissues such as the fascia, where wound healing is delayed [10]. Epinephrine increases the bioavailability of amido amides that are well-established but it has short half-life [11]. Therefore, there is need of an effective medical technique for postoperative pain management.

There are new developments in postoperative pain management. One of that is local infiltration of the surgical wounds with anesthetics, nerve blocker, and NSAID combination. It has been demonstrated that infiltration of the local anesthetics (ILA) decreased postoperative pain by inhibiting transmission of noxious impulses at the site of the wound. Although there have previously been, questionable results reported to ILA to surgeries. However, recent reports have demonstrated that significant effects of ILA in postoperative pain management [12]. Local

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infiltration of the surgical wounds is a simple, safe, and inexpensive procedure [13,14]. Moreover, it does not require the assistance of an anesthesiologist [15]. Surgeons, oneself can do the wound infiltration during closure of the surgical incision to reduce acute postoperative pain. However, despite the widespread use of ILA, there is relatively limited evidence for the optimal combination of drugs used [16]. ILA was the first used in Australia for orthopedic surgery. It could also be applied by continuous wound perfusion via a catheter or pulsatile local anesthetic preparations to the wound. In local infiltration of the surgical wounds, a local anesthetic agent like levobupivacaine, bupivacaine, ropivacaine has been reported to have significant effects on wound healing in animal [17].

Levobupivacaine is the levorotatory enantiomer (S75-R25) form of bupivacaine [8]. Ibuprofen inhibits the production of cyclooxygenase (COX)-1 and COX-2 enzymes and prevents the sensitization of pain receptors at the site of injury [3].

The primary aim of the research was to reduce effectively post-operative pain after laparotomy surgery within a brief period of the time. The secondary endpoint of the finding was to get healing of the surgical wound with good tensile strength.

2. Materials and methods

2.1. Materials

Levobupivacaine, epinephrine, oxygen, and isoflurane were purchased from Neon Lab, India. The wokadine® solution was purchased from Wockhardt, Mumbai, India. Water for injection was purchased from Nirlife, Ahmedabad, India. Veet® cream was purchased from Reckitt Benckiser, India, Pvt. Ltd.

2.2. Ethical statement

The laparotomy procedure was adopted to evaluate the post-operative pain [18]. The study was approved by the institutional animal care and use committee of Sunrise University Alwar, Rajasthan, India, and guidelines of institutional animal ethics committee (IAEC) New Delhi, India were followed [19].

2.3. Prior sample size

The parallel design was used for the study. There were randomly divided into two groups as reported in Table 1. The prior sample size was calculated using OpenEpi 3.01-English, software, Epidemiologic Statistics for Public Health, US. The sample size was found to be 6 with criteria of population size (for finite population correction factor or fpc) (N): 6, hypothesized percentage frequency of outcome factor in the population (p): 95 ± 5%, confidence limits as percentage of 100 (absolute ± %) (d): 5%, and design effect (for cluster surveys): 1. The flowchart of enrollment, allocation, follow-up, and analysis of randomized rats for the experimental study is represented in Fig. 1. There was a washout period of 15 days between vehicle group and treatment group. The first experiment was performed with the vehicle group.

Table 1
Randomization of groups in the study.

Group	Numbers of rats (n)	Treatment
1 (Vehicle)	6	Water for injection
2 (Treatment)	6	0.3% w/v Le, 2 mg/mL Ib, 8 mg/mL Ep

All rats had performed the sensitivity test prior study.
Formaldehyde smoke fumigation method was adopted for sterilization.
Mode of administration for both groups was infiltration of the surgical wound with 50 µL solution.
Le: Levobupivacaine, Ib: ibuprofen, Ep: epinephrine.

2.4. Preoperative preparation

There were 6 rats (267–310 g) of either sex acquired from the animal house of Sunrise University Alwar, Rajasthan, India, maintained on rodent pellets (Pedigree, India) and tap water, housed in pairs in stainless still cages (Remi equipment, Mumbai, India) at a controlled temperature, relative humidity and exposed to a 12 h/12 h light/dark rotation.

Anesthesia was induced in a plexiglass induction chamber (Remi equipment, Mumbai, India) and 2% isoflurane carried in oxygen (1.3 L/ min). It was continued until the loss of the corneal reflex and extremity drawing a response. The hairs of the rat were subjected to remove by Veet® cream. The incision area was cleaned with a wokadine® solution and covered with a sterile drape (Win Surgical, Rajasthan, India). The surface was wiped dry with sterile gauze (Sterling Surgical, Mumbai, India) after six min [20].

2.5. Laparotomy procedure

An average 3 cm incision was made through the skin, fascia, and abdominal muscle by using a sterile scalpel (Sterling Surgical, Mumbai, India) under sterile conditions (Formaldehyde smoke fumigation method was adopted). Muscle wall and the surface of wounds were closed by using sterile suture (Nylon 4.0, Roche, Switzerland) [18]. The laparotomy procedure was performed in the early morning before rats were started feeding.

2.6. Intervention

The incisions were infiltrated with water for injection in the vehicle group. The incisions were infiltrated with three-drug combination of levobupivacaine, ibuprofen, and epinephrine in the treatment group. Then the tissues were closed with surgical suture (Roche, Switzerland) under sterile conditions.

2.7. Rodent grimace scale (GS) scoring method

GS Scorings is used for assessment of pain. It is based on facial expressions of rats related to pain [21,22]. One video camera (HD 1080P 24MP 16X Digital Zoom Video, Besteker, US) was put opposite to cages of the rats. Three video images were noted for each response. The best image was considered for the assessment of pain. The pain score was recorded as 0: absent, 1: mild, 2: mild, 3: moderate, and 4: severe. The observer did not record the images when rats were sleeping and snoozing.

2.8. Histopathological examination

Histopathological studies were carried out after three weeks of treatment for better interpretation [23]. The sutured skin sample was taken from a rat. Then put in 10% formalin (Oxford, Mumbai, India) for fixation purpose. The skin after one day stained with hematoxylin and eosin dye (Oxford, Mumbai, India) and was shown under the light microscope (3N, Olympus, India) with 10× and 45× magnifications [23]. The pathologist blind for the study was considered for the assessment of regeneration of skin. The in-house technique was adopted for assessment of the progress of angiogenesis process was as per Table 2 [24].

2.9. Tensile strength measurement

Tensile strength measurement was performed after three weeks of the treatment. Wound length was measured using a scale of digital tension meter (DX, Remi equipment, Mumbai, India). Muscles were stretched while applying a force. Application of the force continued until a tear was developed anywhere along the incision and the value