ORIGINAL ARTICLE, MEDICINE

Analgesic Effect of Local Infiltration of the Surgical Wound Containing Levobupivacaine, Ibuprofen, and Epinephrine in Rats Undergoing Laparotomy

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Received: 24 May 2017

Accepted: 28 Aug 2017

Published Online: 30 Oct 2017

Published: 30 June 2018

Key words: epinephrine, ibuprofen, laparotomy, levobupivacaine, rodent grimace scales

Citation: Korat PS, Kapupara P. Analgesic effect of local infiltration of the surgical wound containing levobupivacaine, ibuprofen, and epinephrine in rats undergoing laparotomy. *Folia Med (Plovdiv)* 2018;60(2): 254-60.
doi: 10.1515/folmed-2017-0096

Background: Inappropriate use of drugs and their combinations for analgesics has made it difficult to determine the optimal drug combinations for pain management.

Aim: To reduce postoperative pain effectively and safely.

Materials and methods: Laparotomy was performed in an adult rat under iso-flurane anesthesia. During surgery, the surgical wounds were infiltrated with 50 μ L solution containing 0.3% w/v levobupivacaine, 2 mg/mL ibuprofen, and 8 mg/mL epinephrine (treatment group) over the sutured muscle wound before skin closing, and compared to infiltration of that of the normal saline (vehicle group). The 10-fold higher dose of the same combination of medications was injected systemically as a control. Postoperative pain assessed by rodent grimace scales scoring. One-way ANOVA following Dunnett multiple comparisons test was used at 95% of confidence level.

Results: There was decreased pain for the treatment group ($p = 0.025$, $q = 4.527$) and the control group ($p = 0.031$, $q = 4.178$) only 24 h after the end of the successful infiltration. The rodent GS scale scoring showed the fall in pain was started within three hours post-surgery in the treatment group. There was decreased pain in the treatment group ($p = 0.048$, $q = 3.527$) and the control group ($p = 0.043$, $q = 3.891$) only as compared to vehicle group 24 h after the end of the successful infiltration.

Conclusion: The infiltration of the surgical wound with levobupivacaine, ibuprofen, and epinephrine combination was effective in the healing of wounds after laparotomy.

BACKGROUND

Pain is the stress response agent and can result in widespread inflammation, increased catabolism, and poor wound healing.¹ Physiologically, pain serves to alert individuals to tissue damage and to prevent further harm.² Traditionally used methods for management of postoperative pain are epidural analgesia, peripheral nerve blocks, and parenteral or spinal opioids. Although epidural analgesia is efficacious with rare side-effects, major complications have questioned its routine use, specifically in the elderly³ because of technical problems.⁴ Peripheral nerve blocks provide good analgesia, but in order to effectively control postoperative pain, it may be necessary to block the femoral, lateral cutaneous, and obturator nerves⁵, which can be technically

demanding, time-consuming and may result in a residual motor block, and limited by the duration of action of local anesthetics.⁶ Parenteral and spinal opioids are effective in postoperative pain control with minor but troublesome side-effects in the objects. Moreover, opioids are useful in mitigating the sensation of pain but provide no benefits to the disease process. However, their use is often limited by adverse effects including respiratory depression, sedation, allergic reactions, and gastrointestinal events.⁷ Laparotomy is used as a representative surgery that produces spontaneous pain and used to determine the efficacy of common analgesics intra-operatively or prophylactically for postoperative pain management.⁸ Surgeons can use local infiltration of the surgical wound medical technique during closure

of the surgical incision to reduce acute postoperative pain. Local infiltration of the surgical wounds is a simple, safe, and inexpensive procedure.^{9,10} A few studies showed that local infiltration of levobupivacaine reduces the intensity of postoperative pain.¹¹ Ibuprofen provides advantages of the prolonging of pain relief conditions.² Epinephrine (adrenaline) facilitates noradrenergic transmission by stimulating prejunctional β -adrenoceptors.¹²

The objective of the study was to explore the effect of local infiltration containing the three drugs combination of levobupivacaine (long-acting local anesthetic), ibuprofen (NSAID), and epinephrine (vasoconstrictor) into the tissue around the surgical wound to achieve satisfactory pain control against the normal saline.

AIM

PRIMARY AIM

The primary aim of the study was to reduce effectively and safely postoperative pain.

SECONDARY ENDPOINT

To get significant differences for the rodent grimace scales between treatment group and normal saline at 24 h after the end of the successful treatment.

MATERIALS AND METHODS

MATERIALS

Normal saline was purchased from Nirlife, Ahmedabad, India. Levobupivacaine was gift sample and epinephrine was purchased from Neon lab, India. Ibuprofen was purchased from FDC, India.

METHODS

The study was approved by the institutional animal care and use committee of Sunrise University Alwar, Rajasthan, India, under protocol number of SU/COI/RP/15/16. The guidelines of institutional animal ethics committee (IAEC) New Delhi, India were followed.¹³ There were 24 rats of either sex (265–315 g) acquired from the animal house of Sunrise University Alwar, Rajasthan, India, maintained on rodent pellets and tap water, housed in pairs in stainless still cages (Remi equipment, Mumbai, India) at a controlled temperature, relative humidity, and exposed to a 12h/12h light/dark rotation. The parallel design was used for the study. The animals were randomly divided into 4 groups of 6 rats each. The participation, enrollment, allocation, follow-up, and analysis of the rats in the rodent grimace scales scoring experimental was presented

in a flowchart (**Fig. 1**).

Anesthesia was induced in a plexiglass induction chamber (Remi equipment, Mumbai, India) and 2% isoflurane carried in oxygen (1.1 L/ min, Neon lab., India). 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 (Reckitt Benckiser, India, Pvt. Ltd). The incision area was clean with a Wokadine® solution (Wockhardt, Mumbai, India) and covered with a sterile drape (Win Surgical, Rajasthan, India). The surface was wiped dry with sterile gauzes (Sterling Surgical, Mumbai, India) after 6 min.¹⁴ A 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).

INTERVENTIONS

The incisions were infiltrated with 50 μ L normal saline in the vehicle group. The incisions were infiltrated with 50 μ L solution containing three drug combination of 0.3% w/v levobupivacaine, 2 mg/ mL ibuprofen, 8 mg/ mL epinephrine in the treatment group. The same dose of the three-drug combination was applied intra-peritoneally in systematic circulation in group 3. The 10-time higher dose of the treatment group of the three-drug combination was applied intra-peritoneally in the control group. Then the tissues were closed with surgical suture in sterile conditions.

THE RODENT GRIMACE (GS) SCALES SCORING METHOD

GS Scorings is used for assessment of pain. It is based on facial expressions of rats related to pain.^{15,16} One video camera (3CCX, Canon, China) 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 (**Table 1**). The observer did not record the images when rats were sleeping, snoozing.

STATISTICAL ANALYSIS

The one-way ANOVA (Microsoft Excel 2013, Microsoft Raymond USA) followed by the Dunnett multiple comparisons test (InStat Statistica, GraphPad Software, Inc., CA, USA, considering $q > 2.561$ as significant) was used to show significant differences of GS scales between baseline and at the end of the successful study of groups and between groups at 24 h after the end of the successful infiltration. The difference was considered significant at 95%

Figure 1. The rodent grimace scales scoring experimental study flow chart.

of confidence level.¹⁷

RESULTS

One control was lost during the follow-up period for evaluation. There was no decrease in pain 24 h after the end of the successful infiltration of the surgical wound for vehicle group ($p = 0.768$) and group 3. However, there was a decrease in pain for treatment group ($p = 0.025$, $q = 4.527$) (**Fig. 2**) and control group ($p = 0.031$, $q = 4.178$) 24 h after the end of the successful infiltration (**Table 2**). The rodent GS scale scoring showed the fall in pain started within three hours post-surgery in the treatment group as compared to the vehicle group. There

was decreased pain in treatment group ($p = 0.048$, $q = 3.527$) (**Fig. 3**) and control group ($p = 0.043$, $q = 3.891$) as compared to the vehicle group 24 h after the end of the successful infiltration. There was no decrease in pain in group 3 as compared to vehicle group 24 h after the end of the successful treatment ($p = 0.3632$).

DISCUSSION

The present study was for laparotomy surgery on a rat for assessment of postoperative pain. The current clinical studies findings on the local infiltration of the surgical wounds appeared to be on the visual analog scale and human subject.^{11,18} As the cocktail combination (levobupivacaine, ibuprofen, and epinephrine) was used the very first time for local infiltration of the surgical wound, the animal experimental study was preferred. With respect to the subject of the experimental study, the finding was a safety measure to evaluate results.

The current study used 4 groups of 6 rats each. The study did not use the large sample size to overcome β -errors for evaluation of finding.¹⁹ Moreover, sample size could not be the issue, that the real goal is to design a high-quality study.²⁰ The study

Table 1. The measures of scale for GS scoring method

Score	Assessment
0	Absent
1	Mild
2	Mild to moderate
3	Severe

Table 2. Results of rodent GS scoring

Group	Interventions	Level (n)	Score of rodent GS scoring, *N (%)			
			0	1	2	3
1 (Vehicle group)	Infiltrated with 50 µL NS	BL (6)	0(0)	0(0)	1(17)	5(83)
		EP (6)	0(0)	0(0)	2(33)	4(67)
		p-value		0.768		
		q-value		1.123		
		At EP with respect to vehicle group				
2 (Treatment group)	Infiltrated with 50 µL solution containing 0.3% w/v Leb, 2 mg/ mL Ibp, 8 mg/ mL Epn	BL (6)	0(0)	0(0)	0(0)	6(100)
		EP (6)	5(83)	1(16)	0(0)	0(0)
		p-value		0.025		
		q-value		4.527		
		At EP with respect to vehicle group				
Group 3	Intra-peritoneal application of 50 µL solution containing 0.3% w/v Leb, 2 mg/ mL Ibp, 8 mg/ mL Epn	BL (6)	0(0)	0(0)	1(17)	5(83)
		EP (6)	0(0)	0(0)	1(17)	5(83)
		p-value		N/A		
		q-value		N/A		
		At EP with respect to vehicle group				
4 (Control group)	Intra-peritoneal application of 50 µL solution containing 3% w/v Leb, 20 mg/ mL Ibp, 80 mg/ mL Epn	BL (6)	0(0)	0(0)	1(17)	5(83)
		EP (5)	1(17)	5(83)	0(0)	0(0)
		p-value		0.031		
		q-value		4.178		
		At EP with respect to vehicle group				

BL: before treatment, EP: 24 hrs after the end of the successful infiltration of the surgical wound.

NS: Normal saline, Leb: levobupivacaine, Ibp: ibuprofen, Epn: epinephrine

The *p*-value for one-way ANOVA and the *q*-value for the Dunnett multiple comparisons test.

The *n* value indicated number of rats in group

*All data were presented as Numbers of rats (Percentage)

N/A: not applicable

also used the Dunnett multiple comparisons test for the significant differences between the parameters as compared to the other *post hoc* tests.²¹ With respect to prior sample size calculations and the statistical analysis method adopted for the study,

the finding was justified for the small sample size for the significant physiological effect.

The study reported the decrease in postoperative pain started within three hours of surgery in the treatment group. The current studies for the local

Figure 2. Results of rodent GS scoring for the treatment group. Significant difference for GS scores between baseline and at 24 h after the end of the successful infiltration of the surgical wound ($p = 0.025$, $q = 4.527$).

infiltration of the surgical wounds are with 50 μ L bupivacaine, 0.75% w/v ketorolac, and 6.0 mg/mL of dexamethasone after laparotomy in rats.²² There is a significantly high dose of these drugs required for the therapeutic effect. With respect to the doses and dosages of the selected cocktail combinations in the study, the current findings provide the physiological effect in very low dose.

A limitation of this study, for example, is that it was evaluated for the abdominal and facial scars only. The study was not evaluated for arthroscopic surgery, tonsillectomy, and colostomy surgical procedures. The literature review was limited to the English language published articles and books only. The study failed to concentrate on non-English published materials.

CONCLUSION

The experimental study suggested that local infiltration of the surgical wound with levobupivacaine, ibuprofen, and epinephrine combination was effective in the postoperative pain management. Further larger animal study is needed to state the hypothesis strongly.

CONFLICT OF INTEREST

None.

Figure 3. Results of rodent GS scoring at 24 h after the end of the successful infiltration of the surgical wounds for vehicle group and treatment group. Significant difference for GS score between treatment group and vehicle group ($p = 0.048$, $q = 3.527$).

ACKNOWLEDGEMENTS

The authors thank all the individuals who took part in these studies and other healthcare providers, technicians, and administrative staff who have enabled this work to be carried out. The authors especially thank Sunrise University, Alwar, Rajasthan, India for providing all the necessary facilities for carrying out my research work.

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Анальгетический эффект локальной инфильтрации хирургической раны, содержащей левобупивакаин, ибупрофен и эпинефрин у крыс, подвергнутых лапаротомии

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Введение: Неправильное использование лекарств и их комбинации в качестве анальгетиков затрудняет определение оптимальных комбинаций лекарств для обезболивания.

Цель: Эффективное и безопасное лечение послеоперационной боли.

Материалы и методы: Лапаротомия была проведена у взрослой особи крысы под изофлурановой анестезией. Во время анестезии в хирургические

Дата получения: 24 мая 2017
Дата приемки: 28 августа 2017
Дата онлайн публикации: 30 октября 2017
Дата публикации: 30 июня 2018

Ключевые слова: эпинефрин, ибупрофен, лапаротомия, левобупивакаин, шкала мышинных гримас для определения боли (rodent grimace scales)

Образец цитирования:
Korat PS, Kapurara P. Analgesic effect of local infiltration of the surgical wound containing levobupivacaine, ibuprofen, and epinephrine in rats undergoing laparotomy. Folia Med (Plovdiv) 2018;60(2): 254-60.
doi: 10.1515/folmed-2017-0096

раны инфильтрировали 50 μ L раствора, содержащего 0.3% w/v левобупивакаина, 2 мг/мл. ибупрофена и 8 мг/мл. эпинефрина (экспериментальная группа) через зашитую мышечную рану перед закрытием кожи и сравнивали с инфильтрацией нормального физиологического раствора (рабочая группа). В 10 раз более высокую дозу той же комбинации лекарств вводили системно в качестве контроля. Послеоперационная боль оценивалась по шкале мышинных гримас (rodent grimace scales). Был использован однофакторный дисперсионный анализ (ANOVA) после множественного сравнительного теста Dunnett 95% достоверности.

Результаты: Уменьшилась боль в экспериментальной группе ($p=0.025$, $q=4.527$) и в контрольной группе ($p=0.031$, $q=4.188$) только через 24 часа после окончания успешной инфильтрации. Результаты по шкале мышинных гримас GS показали, что спад боли начался в течение трёх часов после операции в экспериментальной группе. Уменьшилась боль в экспериментальной группе ($p=0.048$, $q=3.527$) и в контрольной группе ($p=0.043$, $q=3.891$) по сравнению с рабочей группой через 24 часа после окончания успешной инфильтрации.

Заключение: Инфильтрация хирургической раны с помощью комбинации левобупивакаина, ибупрофена и эпинефрина оказалась эффективной при заживлении ран после лапаротомии.