Submitted: 13 September, 2024 Accepted: 22 November, 2024 Published: 08 June, 2025 DOI:10.22514/sv.2025.029

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ORIGINAL RESEARCH

Effects of bupivacaine wound infiltration and transversus abdominus plane block on healing and postoperative adhesion of colonic anastomosis in rats

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Abstract

Background: Effective pain management following surgery is crucial for facilitating a prompt and successful recovery. Epidural anesthesia, peripheral nerve blocks, and local infiltration anesthesia are used for this purpose. The main objective of this study is to evaluate the impact of bupivacaine infiltration anesthesia and transversus abdominus plane (TAP) block, both commonly used for postoperative pain management, on colon anastomosis. This study also examined whether it affects abdominal incision wound healing and postoperative adhesions. **Methods**: The current study involved 21 rats. The rats were divided into three groups, with seven rats in each. In this study, the participants were divided into three groups: Group C, I and T. Group C underwent laparotomy followed by colon anastomosis. Group I received laparotomy, colon anastomosis and bupivacaine infiltration. Group T underwent laparotomy, colon anastomosis, and transverse abdominus oblique muscle intervention. Wound tension strength (WTS), anastomosis burst pressure (ABP), and colon hydroxyproline levels were measured. The macroscopic adhesion score was detected. Histopathological examinations of the colon and wound were conducted. **Results**: The mean WTS for Group T was statistically significantly higher than Group C ($p = 0.035$). Wound Histopathology Score (WHS), Colon Histopathology Score (CHS), Colon hydroxyproline (COHP), and ABP values of the rats in the groups (respectively; $p = 0.154/0.538/0.999/0.178$). **Conclusions**: There are limited studies in the literature showing the effects of central blocks on direct colonic anastomosis. However, no studies were found on the effects of TAP block and local infiltration anesthesia (LIA) on postoperative colonic anastomosis. This study found that TAP block and LIA did not significantly affect colon anastomosis. Additionally, the TAP block demonstrated a statistically significant favorable impact on the healing of abdominal wounds. Our study suggests that TAP block may be more effective than LIA as a postoperative analgesic modality for colon surgery in terms of abdominal wound healing.

Keywords

Transversus abdominus; Plane block; Infiltration anesthesia; Bupivacaine; Colon

1. Introduction

Colorectal surgery is a leading procedure within the field of major abdominal surgeries [1]. Anastomotic leakage is a serious problem associated with an increased risk of morbidity, mortality, and prolonged hospitalization [2]. It is well-established that surgical stress triggers various neuroendocrine and metabolic alterations. The phenomenon of surgical stress can be divided into two distinct phases. During the initial stage, there is an increase in the levels of adrenocorticotrophic hormone (ACTH) and cortisol. Following this, cytokines are released, and acute-phase reactants are synthesized [3]. Corticosteroids (CS) are known to impair multiple stages of the wound healing process, including collagen synthesis and the formation of granulation tissue. In addition, it has been observed that CS exhibits an immunosuppressive effect by modulating various cellular processes. Hence, several studies have shown that the administration of corticosteroids increases the likelihood of perianastomotic abscess and peritonitis, adversely affecting the healing process [4, 5]. Additionally, postoperative pain has been found to increase cortisol levels, a catabolic hormone, by augmentation of adrenocortical and sympathetic responses [3, 6]. Thus, the increase in cortisol

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secretion caused by postoperative pain may further increase the risk of anastomotic leakage by negatively influencing colon anastomosis.

The existing literature presents inconclusive findings regarding the impact of local anesthetic applications on postsurgical cortisol levels. However, several studies have indicated that administering intrathecal bupivacaine can mitigate the rise in plasma cortisol levels [7, 8]. This suggests that improving postoperative pain management could lead to better outcomes in colon anastomosis. Moreover, the existing literature also shows conflicting findings regarding the impact of epidural analgesia using local anesthetics on colon anastomosis [9, 10]. Effective pain management following surgical procedures is important for facilitating a prompt recovery. Techniques such as epidural anesthesia, plane blocks and infiltration anesthesia are widely employed for this purpose [11, 12]. Bupivacaine, a commonly used local anesthetic agent, plays a key role in providing postoperative analgesia [2].

The findings from various studies investigating the impact of epidural bupivacaine on colon anastomosis have yielded inconclusive results [10]. Furthermore, despite the widespread use of epidural anesthesia as a reliable method for pain relief, it is important to acknowledge the potential for significant complications [13]. Currently, there is a lack of comprehensive academic research on examining the impact of bupivacaine infiltration anesthesia and transversus abdominis facial plane (TAP) block administration on colon anastomosis.

The main objective of this study is to evaluate the impact of subcutaneous bupivacaine wound infiltration anesthesia and TAP block, both commonly employed for postoperative pain management, on healing and postoperative adhesion of colonic anastomosis in rats. Furthermore, this study aimed to investigate the potential impact of these interventions on the wound-healing process in abdominal incisions and the occurrence of postoperative adhesions.

2. Material and methods

2.1 Animal housing and care

This study was conducted using 21 male Sprague rats, with an age range of 16 to 20 weeks and an average weight of 380 ± 30 grams. During the experiment, the rats were housed in metallic cages at a consistent room temperature of 21 ± 2 °C and a relative humidity range of 40–60%. The rats were subjected to a 12-hour light-dark cycle, with each rat housed individually per cage. Their diet consisted of standard rat food, and they had access to tap water. Routine cage maintenance was carried out with daily inspections. Throughout the investigation, all rats were subjected to humane treatment in compliance with the guidelines outlined in the "Guide for the Care and Use of Laboratory Animals". All surgical procedures were performed under anesthesia, which was induced by intraperitoneal (IP) injection of Ketamine Hydrochloride at 80 mg/kg and Xylazine Hydrochloride at 10 mg/kg IP [2].

2.2 Experimental design

Group C (Control) (n = 7): Laparotomy + colon anastomosis + subcutaneous injection 3 mL saline.

Group I (Infiltration) (n = 7): Laparotomy + colon anastomosis + 2 mg/kg/3 mL bupivacaine infiltration of the planned incision site.

Group T (Transverse abdomino-oblique plane block) (n = 7): Laparotomy + colon anastomosis + bupivacaine (2 mg/kg/3 mL) injection into the transverse abdomino-oblique muscle.

2.3 Surgical procedures

Under anesthesia, the weights of all rats were measured and recorded after 8-hour fasting. Intravenous access was established through the tail vein using a 26-gauge angiocatch while under anesthesia. Prophylactic administration of cefazolin sodium at 30 mg/kg/mL and metronidazole at 7.5 mg/kg/mL was conducted 30 minutes before the laparotomy procedure [2]. Following the removal of hair from the abdominal region of each rat and the subsequent cleansing of the surgical area using povidone-iodine within the designated operating room, a midline abdominal incision measuring 3 cm in length and penetrating through all layers of tissue was carried out across all experimental groups.

A single-layer colon anastomosis was performed using a 5/0 round vicryl suture after a full-thickness transection of the transverse colon. The abdominal midline incision was closed using a 4/0 silk suture. Following the closure of the abdominal midline, Group C rats received a subcutaneous injection of 3 mL isotonic solution along the incision line. Group I rats received an injection of 2 mg/kg/3 mL bupivacaine along the incision line, and Group T rats received the same dosage of bupivacaine injected into the transversus abdominis muscle planes.

2.4 Postoperative care and tissue collection

Each group of rats was administered oral paracetamol at a dosage of 20 mg/kg/day for analgesia. The rats received routine wound care. All rats were weighed again under anesthesia on the seventh postoperative day, and laparotomies were performed through midline abdominal incisions. After removing the central one-third portion of the incision line, measuring 2 cm to the right and left, it was carefully collected and stored in Eppendorf tubes. The samples were then frozen at a temperature of −80 °C for wound tensile strength (WTS) analysis. A 0.5 × 0.5 cm surgical cut in the upper one-third region of the incision was immersed in Eppendorf tubes containing a 10% formaldehyde solution for histological examination.

2.5 Assessment of anastomotic integrity

A macroscopic adhesion score (MAS) was recorded during the laparotomy procedure. The segment of the colon that underwent anastomosis, including 2–3 cm proximal and 2–3 cm distal to the anastomosis site, was excised to assess the burst pressure of the anastomotic area. Subsequently, fifty percent of the acquired tissue samples, including a 0.5 cm segment proximal and distal to the anastomosis line, were preserved in formaldehyde for subsequent histopathological analysis. The remaining fifty percent was stored at a temperature of −80 °C for hydroxyproline levels measurement. All animals were

sacrificed via intracardiac puncture to obtain tissue samples.

2.6 Wound tensile strength measurement

A uniaxial tensile testing system (Instron 3382 test frame) was used to conduct the tests. The apparatus described in the study allows for the investigation of tissue samples at a strain rate of $10^{-3} \cdot s^{-1}$. Tensile strength was determined by measuring the force required to cause tearing or fracture in the tissue samples, and this force was subsequently used to calculate the tensile strength. To assess the repeatability of the findings, multiple trials, ranging from two to three, were conducted on companion specimens for each case. The data presented in this study were recorded on the Newton (N) site [2].

Macroscopic adhesion scoring: No adhesion: 0 points, single band: 1 point, two bands: 2 points, multiple bands: 3 points, direct visceral adhesion; fusion: 4 points [14].

2.7 Anastomosis burst pressure measurement

The anastomosis line was located, and a 5 cm segment of the intestine, encompassing 2 cm proximal and 2 cm distal to the anastomosis area, was resected using the method used in the study by Kesici *et al*. [2]. After the 18-gauge (18 G) intraluminal catheter was placed in the intestinal lumen, one end was sutured and the other end was tied. A three-way cannula was attached to the catheter's end, with one outlet connected to the intraluminal side and another to a manometer. Methylene blue diluted with saline was applied with an infusion pump at a rate of 6 mL/min. The occurrence of blue-colored fluid emergence or a sudden pressure drop was noted as the burst pressure and recorded in millimeters of mercury (mmHg) [2].

2.8 Histopathological examination

Following the standard protocol for tissue examination, tissue samples obtained from the anastomosis and incision sites were preserved in paraffin blocks to facilitate subsequent histopathological assessment. Tissue sections measuring 4–5 microns in thickness were stained with hematoxylin and eosin and then examined under a light microscope. In the present study, the inflammatory cell types observed in the anastomosis and incision area were categorized in a semi-quantitative manner. These cell types included polymorphonuclear leukocytes (PMN), lymphocytes and plasma cells. The classification was based on the density of collagen fibers and the presence of neovascularization, with the following classification scale: −, +, ++ and +++. Furthermore, the assessment of wound healing at the location of the anastomosis and incision was conducted using a scale ranging from 1 to 5, as described in a previous study [15].

2.9 Biochemical method

Wound and colon tissues were homogenized using phosphate-buffered saline (1× PBS, 0.1 mol/L, pH 7.4) solution in a homogenizer. Supernatants were centrifuged at 10,000 × g for 10 min at +4 °C for measurement of wound hydroxyproline (WOHP) and colon hydroxyproline (COHP) levels (Beckman Coulter Allegra® X-30, Indianapolis, IN, USA). Protein concentrations in the supernatant were measured at 595 nm using a commercial kit based on the Bradford method (Coomassie Plus (630), Protein Assay (3160), ThermoFisher Scientific, Waltham, MA, USA). Samples were evaluated by spectrophotometry at a wavelength of 540 nm. Hydroxyproline levels were calculated by comparison with a previously determined hydroxyproline standard curve [2].

2.10 Power analysis

To minimize the number of animals used, in alignment with the 3R principle (Replacement, Reduction, and Refinement), a power analysis was conducted as suggested by "Dogan *et al.* [16]", mean pressure values were assumed to be 95 with a standard deviation of 5 for one group, and 105 with a standard deviation of 5.5 for another. It was statistically determined that 7 experimental animals per group would achieve a power of 0.95 and an error level (alpha) of 0.05.

2.11 Statistical analysis

Descriptive statistics were employed to summarize continuous variables. Median values were provided for parameters unsuitable for normal distribution while mean and standard deviation values were provided for parameters acceptable for normal distribution. The conformity of continuous variables to the normal distribution was investigated using the Shapiro-Wilks test. The Kruskal-Wallis Test was used to analyze the difference between three independent groups of continuous variables that did not conform to the normal distribution. *Post Hoc* pairwise comparisons were conducted using the Mann-Whitney U test with Bonferroni correction for significant comparisons. The association between categorical variables was explored using the Chi-Square test. The difference between three dependent groups of continuous variables not following a normal distribution was studied using the Wilcoxon Signed Rank test. The correlation between continuous variables that did not adhere to the normal distribution was evaluated using the Spearman-Rho correlation coefficient. The level of statistical significance was determined to be 0.05. All analyses were conducted using MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium).

3. Result

Because T3 was found dead in its cage on the fourth post-operative day, only the initial weight was recorded. The determination of Anastomotic Burst Pressure (ABP) values was compromised in rats C2, I5 and T2 due to separation at the anastomosis line during dissection, and in rat C4 due to micro perforation at the anastomosis line. Additionally, the measurement of WTS (Wound Tensile Strength) values was impeded in rats C5 and T6 by tissue decomposition during device placement.

No statistically significant differences were observed in the mean initial and final weights of the groups ($p = 0.879$ and $p = 0.837$, respectively). However, statistically significant decreases in the final mean weights compared to the initial mean weights were observed in Group C ($p = 0.018$), Group

I (p = 0.018), and Group T (p = 0.027). Table 1 presents the mean weights of the rats in each experimental group at both the beginning and the seventh day following the surgical procedure.

No statistically significant differences were observed in the groups' MAS (p = 0.733). The results of the pairwise comparisons indicate that the mean WTS for Group T was significantly higher than the mean WTS for Group C (p = 0.035). No statistically significant differences were observed in the mean WTS values of the other groups when pairwise comparisons were made. Table 2 presents the average values of MAS and WTS for the rats in each experimental group. Furthermore, there was no statistically significant difference observed in the mean values of WHS, Colon Histopathology Score (CHS), Colon hydroxyproline level (COHP), and ABP between the groups of rats (p = 0.154, p = 0.538, p = 0.999, p = 0.178, respectively). Table 3 displays the average WHS, CHS, COHP and ABP values for the rats within each group.

4. Discussion

The primary outcome of the study was anastomotic bursting pressure, and the secondary outcomes were WTS and MAS. No significant difference was found between the two groups in terms of anastomotic bursting pressures. However, WTS was statistically higher in Group T compared to Group C, whereas no significant differences were noted in MAS between the groups.

Colorectal surgery is the most prevalent major abdominal surgical procedure, necessitating the critical importance of effective postoperative pain management to facilitate rapid recovery. Postoperative pain has been found to have the potential to elevate cortisol levels and disrupt the process of wound healing. The potential consequences of this phenomenon include an elevated risk of morbidity and mortality, primarily due to the increased probability of experiencing significant complications such as anastomotic leakage [11]. Current debates persist regarding the optimal approach to postoperative pain management, specifically in the context of major abdominal surgery. Different techniques are utilized to manage postoperative pain, including infiltration anesthesia, plane blocks and central blocks such as epidural, spinal and caudal blocks [11, 17].

Existing literature from both experimental and clinical studies on the impact of central blocks on colorectal surgery yields conflicting conclusions. Concisely, Adanır *et al*. [9] reported a positive effect of epidural lidocaine on colon anastomosis. Conversely, Jansen *et al*. [10] expressed uncertainty regarding the influence of epidural bupivacaine on colon anastomosis. Although epidural anesthesia is widely considered the preferred method for managing postoperative pain in open colorectal procedures, its rare but significant complications necessitate considering alternative techniques such as abdominal plane blocks. The available research comparing abdominal plane blocks to central blocks does not provide sufficient evidence to support the superiority of plane blocks [18].

Based on ongoing investigations, it has been found that local infiltration anesthesia (LIA) provides a high level of pain relief

TABLE 1. Initial and postoperative mean weights of the rats in the groups.

	Group C	Group I	Group T	p
WI				
Mean ± Sd	381.3 ± 24.2	384.4 ± 17.5	385.3 ± 24.8	0.879[1]
Med (min–max)	381 (341–412)	385 (354–405)	380 (361–423)	
LW				
Mean ± Sd	352.4 ± 30.3	358.4 ± 17.0	355 ± 25.6	0.837[1]
Med (min–max)	355 (316–390)	365 (327–377)	343 (334–389)	
p	0.018[3]	0.018[3]	0.027[3]	

[1]*Kruskal Wallis Test;* [3]*Wilcoxon Signed Rank Test.*
WI: Weight initial; LW: Last Weight; Med: Median; Sd: Standard deviation; min: minimum; max: maximum.

TABLE 2. Mean MAS and WTS values of the rats in the groups.

	Group C	Group I	Group T	p
MAS				
Mean ± Sd	0.6 ± 0.5	0.7 ± 0.5	0.5 ± 0.5	0.733[1]
Med (min–max)	1 (0–1)	1 (0–1)	0.5 (0–1)	
WTS (Newton)				
Mean ± Sd	2.1 ± 0.7	2.7 ± 0.7	3.2 ± 0.3	0.035[1]
Med (min–max)	2.1 (1.1–3.2)	2.3 (2.1–3.8)	3.3 (2.7–3.4)	

[1]*Kruskal Wallis Test.*
MAS: Macroscopic Adhesion Score; WTS: Wound Tension Strength; Med: Median; Sd: Standard deviation; min: minimum; max: maximum.

TABLE 3. Mean WHS, CHS, COHP and ABP values of rats in groups.

	Group C	Group I	Group T	*p*
WHS				
Mean ± Sd	6.4 ± 1.1	6.4 ± 1.3	7.5 ± 0.5	0.154[1]
Med (min–max)	7 (5–8)	6 (5–8)	7.5 (7–8)	
CHS				
Mean ± Sd	5.0 ± 0.6	4.4 ± 1.0	4.7 ± 1.4	0.538[1]
Med (min–max)	5 (4–6)	4 (3–6)	5 (3–6)	
CHOP (µg/mL)				
Mean ± Sd	1.7 ± 0.5	1.8 ± 0.6	1.7 ± 0.4	0.999[1]
Med (min–max)	1.5 (1.3–2.5)	1.8 (1.1–2.7)	1.8 (1.2–2.3)	
ABP (mmHg)				
Mean ± Sd	222 ± 13	185 ± 38.3	208 ± 36.3	0.178[1]
Med (min–max)	220 (210–240)	185 (140–240)	200 (160–260)	

[1]*Kruskal Wallis Test.*
WHS: Wound Histopathology Score; CHS: Colon Histopathology Score; CHOP: Colon Hydroxyproline Level; ABP: Anastomosis Burst Pressure; Med: Median; Sd: Standard deviation; min: minimum; max: maximum.

that is comparable to block anesthesia [19]. Successful performance of a transversus abdominis facial plane (TAP) block depends critically on the use of ultrasonography and expertise in ultrasound-guided regional anesthesia. Consequently, the results of various studies conducted in this field have been equally intriguing [17]. However, the existing research does not provide adequate evidence to support the claim that LIA can be a viable substitute for abdominal plane blocks. As a result, the ongoing debate regarding this issue remains yet to be resolved. Previous studies in literature have primarily focused on comparing TAP block and LIA concerning postoperative pain and the need for rescue analgesics [17, 19, 20]. However, comprehensive data regarding their specific effects on direct surgical wound healing is lacking. While there is a theoretical expectation that reducing postoperative pain could potentially enhance surgical recovery, it is widely recognized that empirical evidence is required to support this claim. No studies have specifically investigated the impact of TAP block and local infiltration anesthesia on postoperative colon anastomosis. Given the aforementioned considerations, we believe that the results of this study possess the potential to offer valuable insights into the choice of postoperative pain management strategies employed within the context of clinical practice. Our study's findings suggest that using TAP block and LIA with bupivacaine leads to a statistically insignificant reduction in the mean ABP and CHS. The comparison of two distinct analgesic modalities with the control group did not yield a statistically significant difference in early postoperative abdominal adhesion, as observed in one of the secondary outcomes of our study. This situation may be favorable in scenarios where laparotomy becomes necessary to address anastomotic leakage, which is recognized as a very serious complication that may arise after colon surgery. Furthermore, the observed increase in WTS values in the TAP block group compared to the control group provides statistical evidence supporting the advantageous effects of wound healing in the laparotomy incision region during major abdominal surgeries. This suggests that the use of a TAP block has the potential to mitigate complications associated with early evisceration, particularly in the context of major abdominal surgeries. The limitation of this study is that the study was experimental and basal and postoperative cortisol levels could not be measured.

5. Conclusions

The management of postoperative pain after major abdominal surgery is crucial for facilitating rapid recovery. Ongoing discussions persist regarding the optimal approach to postoperative pain treatment. Despite ongoing debates about the optimal approach to postoperative pain treatment, limited research has addressed the effects of central blocks on direct colon anastomosis. Moreover, studies exploring the impact of transversus abdominis facial plane (TAP) block and local infiltration anesthesia (LIA) on postoperative colon anastomosis are scarce. This study revealed that both the TAP block and LIA did not exhibit a statistically significant effect on colon anastomosis. Furthermore, it was determined that neither modality showed an increase in postoperative adhesion. However, it was found that the TAP block demonstrated a statistically significant positive impact on the healing of abdominal wounds. According to the results of our research, although TAP block does not have an additional advantage in terms of anastomosis in colon surgery, it may be preferred over LIA as a postoperative analgesic modality due to its advantages in wound healing.

6. Key points

1. Postoperative pain management is important for early recovery.
2. Epidural anesthesia is regarded as the gold standard for postoperative pain management.
3. Uncommon serious complications of epidural anesthesia

may occur.

4. Facial Plane blocks and LIA are alternative methods.

5. It is clinically important to reveal the effects of facial plane blocks and LIA applications on direct surgery.

ABBREVIATIONS

WI, Weight initial; LW, Last weight; ABP, Anastomosis burst pressure; MAS, Macroscopic adhesion score; WTS, Wound tension strength; WHS, Wound histopathology score; CHS, Colon histopathology score; COHP, Colon hydroxyproline level; TAP, Transversus abdominal plane; LIA, Local infiltration anesthesia; ACTH, adrenocorticotrophic hormone; CS, Corticosteroids; IP, intraperitoneal; N, Newton; G, gauge; PMN, polymorphonuclear leukocytes; PBS, phosphate-buffered saline; WOHP, wound hydroxyproline.

AVAILABILITY OF DATA AND MATERIALS

All data related to the study are owned by the responsible author and can be shared upon request.

AUTHOR CONTRIBUTIONS

SK—Conception and study design, analysis and interpretation, experimental procedures, data collection, writing the article, critical revision of the article and obtaining funding, literature review. YKK—Experimental procedures, data collections, literature review. SO—Analysis and interpretation, critical revision of the article and obtaining funding, literature review. MSG—Experimental procedures, data collections, literature review. AFM—Experimental procedures, data collections, literature review. KB—All biochemical examination, literature review. MGD—Literature review, critical revision of the article, English editing. Tİ—All histopathological examination, literature review. EMG—All biochemical examination, literature review. UK—Study design, analysis, and interpretation, experimental procedures, data collection, writing the article, critical revision of the article, literature review.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval for this study (Ethical Committee Approval No: 11/01) was provided by the Saglik Bilimleri University, Hamidiye Animal Experiments Local Ethics Committee (Chairperson Prof. Dr. Ulkan Kilic) on 24 November 2022.

ACKNOWLEDGMENT

For technical support, we would like to thank Murat Baydogan, a faculty member of the Istanbul Technical University, Metallurgical and Materials Engineering Department.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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How to cite this article: Sevgi Kesici, Yahya Kaan Karatepe, Sibel Oba, Mahmut Salih Genç, Ahmet Furkan Mazlum, Kubra Bozali, *et al*. Effects of bupivacaine wound infiltration and transversus abdominus plane block on healing and postoperative adhesion of colonic anastomosis in rats. Signa Vitae. 2025; 21(6): 33-39. doi: 10.22514/sv.2025.029.