INTRODUCTION

The main problems in postoperative patients are pain and wound. Wound healing consists of four major complex and dynamic phases. One of the most important phases in wound healing is the transition from the inflammatory to the proliferative phase. Inhibition of the inflammatory phase will cause delays in the transition from the inflammatory phase to the proliferative phase. Ropivacaine infiltration is expected to reduce surgical stress. One of the parameters that can be used to assess wound healing is Vascular Endothelial Growth Factor (VEGF) expression. This study aims to evaluate the infiltration effect of ropivacaine on the expression of VEGF in post-surgical skin defects of Wistar rats.

METHODS

This study was an experimental study using 32 Wistar rats (Rattus norvegicus), which were randomly divided into 2 groups. With a 2 cm full-thickness wound incision, the ropivacaine group was given 0.2% 1 ml of ropivacaine infiltration, while the control group was given NaCl infiltration. Tissue samples were taken on day 5 to calculate VEGF expression through immunohistochemical examination. Data were analyzed using SPSS version 23.0 for Windows.

RESULTS

Assessment of the weight of the Wistar rats did not show a significant difference (p = 0.788). Using the IRS (Immunoreactivity Score), there was a significant difference in VEGF expression (p = 0.033) between the two groups. There were significant differences in the degree of VEGF cells between the two groups (p = 0.001). Analysis of the relationship between treatment and degree of VEGF cells showed a significant relationship (p = 0.005).

Conclusion:

Infiltration of ropivacaine had effects on the expression of VEGF in post-surgical skin defects of Wistar rats.

Keywords:

Immunoreactivity Score, Ropivacaine, VEGF Expression, Wound Healing.

Cite This Article:


ABSTRACT

Background: The main problems of postoperative patients are pain and wound. The healing phases consist of hemostasis, inflammation, proliferation, and remodeling. Postoperative pain causes surgical stress, which disrupts wound healing by delaying the transition from the inflammatory phase to the proliferative phase. Ropivacaine infiltration is expected to reduce surgical stress. One of the parameters that can be used to assess wound healing is Vascular Endothelial Growth Factor (VEGF) expression. This study aims to evaluate the infiltration effect of ropivacaine on the expression of VEGF in post-surgical skin defects in Wistar rats.

Methods: This study was an experimental study using 32 Wistar rats (Rattus norvegicus), which were randomly divided into 2 groups. With a 2 cm full-thickness wound incision, the ropivacaine group was given 0.2% 1 ml of ropivacaine infiltration, while the control group was given NaCl infiltration. Tissue samples were taken on day 5 to calculate VEGF expression through immunohistochemical examination. Data were analyzed using SPSS version 23.0 for Windows.

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Conclusion: Infiltration of ropivacaine had effects on the expression of VEGF in postsurgical skin defects of Wistar rats.

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acclimatization period. The research object was then divided into two groups (consisting of 16 rats each): an intervention (ropivacaine) group and a control group. The 2 cm full-thickness wound was made in the rat’s abdomen (Figure 1A and 1B) and followed up until 5 days of healing (Figure 1C). The ropivacaine group was given 0.2% 1 ml of ropivacaine infiltration, while the control group was given NaCl infiltration. 0.9% 1 ml. Tissue samples (Figure 1D-1F) were taken to calculate VEGF expression through immunohistochemical examination.

**VEGF expression examination and interpretation**
The VEGF expression experiment was performed using a commercially available VEGF kit (Anti-VEGF/VEGFA Antibody Picoband; Boster Biological Technology) following the manufacturer’s instructions. One expert pathologist performed the interpretation. The intensity of VEGF expression was viewed using Nikon Eclipse and NIS Element F 4.60.00 64-bit software. Figure 2 shows the result of the immunohistochemistry. The Immunoreactivity Score (IRS) was calculated by multiplying the intensity level and degree of VEGF cell expression as previously described.

**Statistical analysis**
The statistical analysis was performed using the SPSS statistical software package (version 23.0; IBM Corp., Armonk, NY, USA). Discrete variables were tested using the Chi-square test. Statistical significance was determined when the P value was less than 0.05.

**RESULTS**

**Sample characteristics**
Researchers examined data pertaining to the test animals used in this investigation. Mice that meet the requirements for this study’s test animals should be between 150 and 200 grams in weight and between 3 and 4 months old (12 to 16 weeks). The control group’s maximum and minimum body weights were 200 grams and 150 grams, respectively. The control group’s average body weight was 178.1 grams, with a median of 180 grams and a standard deviation of 16.7 grams. There was no discernible difference in body weight between the two test groups, according to the Mann-Whitney analysis (P = 0.788). The characteristics of the animal samples are shown in Table 1.

**Analysis of VEGF expression**
In this study, we examined the percentage of positive VEGF cells, the percentage degree of positive VEGF cells, and the IRS (Immunoreactivity Score) score. In this investigation, all materials displayed reactions to staining of varied intensities and were thoroughly stained. Based on the degree of percentage of positive VEGF cells grouped into 5: no positive cells (score: 0), ≤10% positive cells (score: 1), 11 – 50% positive cells (score 2), 51 – 80% positive cells (score 3) and >80% positive cells (score: 4). In this study, 1 sample was obtained with a percentage of ≤10% (1/32; 3.1%), 19 samples with a percentage of 11 – 50% (11/32; 59.4%), 10 samples with a percentage of 51 - 80% (10/32; 31.3%), and 2 samples (2/32; 6.3%) with a percentage of >80% (Table 2).

In addition to being grouped by degree of percentage, researchers also grouped VEGF expressions based on the frequency of each positive VEGF cell
percentage value. This assessment found that the most were the 40% percentage values that appeared in 6 samples (6/32; 18.8%), followed by the percentage value of 20% and 30% (5/32; 15.6% for both). The percentage value that appears least often is the 10% percentage value, which is only obtained in 1 sample (1/32; 3.1%). Researchers also assessed the IRS score obtained from the intensity of staining multiplied by the degree percentage of positive VEGF cells. Based on IRS scores, 2 samples with IRS scores of 2 (2/32; 6.3%), 12 samples with IRS scores of 4 (12/32; 37.5%), 13 samples with IRS scores of 6 (13/32; 40.6%), 3 samples with IRS scores of 9 (3/32; 9.4%), and 2 samples with IRS scores of 12 (2/32; 6.3%) (Table 2).

The comparison between control and experimental groups
We analyzed the comparison of data distribution between the two groups tested in this study, namely the control group and the treatment group. Because all research variables examined in this study were abnormally distributed, non-parametric analysis was used with the Mann-Whitney test to assess the data distribution on each variable.

There was no significant difference in the intensity variable between the control group and the treatment group (P = 0.714). Meanwhile, based on percentage variables, it was found that there was a significant difference between the control group and the treatment group (P = 0.006). Based on the percentage degree variable, there was a significant difference between the control and treatment groups (P = 0.001). Finally, in the IRS score variable, a significant difference was obtained between the control and treatment groups (P = 0.033) (Table 3).

Association between intensity and degree of VEGF staining with ropivacaine infiltration
We also analyzed the relationship between treatment and various variables used in this study. The Chi-Square analysis results showed no significant relationship between intensity and ropivacaine infiltration treatment in test animals (P = 0.710). Furthermore, the same analysis found a significant relationship between the percentage degree of VEGF and the treatment of ropivacaine infiltration in test animals (P = 0.005).

DISCUSSION
Postoperative patients are faced with several problems, including postoperative wounds and pain. Wounds can cause complications that cause morbidity and even mortality for patients. There was a significant difference between the control group and the ropivacaine group, where the percentage of VEGF cells stained was higher in the ropivacaine group. In
addition, from the statistical analysis, it was found that there was a relationship between ropivacaine infiltration and the percentage degree of positive VEGF. This result is consistent with a study conducted by Subiantoro A et al., which stated that ropivacaine had a beneficial effect on superficial wound healing in Wistar rats. In his research, the Allred Scoring System was used as a parameter for VEGF expression in tissues. The Allred scoring system is obtained from the proportion score results multiplied by the stained cells’ intensity. The results found that administration of ropivacaine can shorten the inflammatory phase, thereby accelerating wound healing and forming new blood vessels.9,10

The expression of VEGF was further assessed using the Immunoreactivity Score (IRS). This assessment using the IRS results from the staining intensity score multiplied by the percentage degree of positive VEGF cells on readings under a light microscope.10 There was a significant difference between the IRS (immunoreactivity score) in the ropivacaine group and the control group. Clinically, these results show that ropivacaine infiltration affects wound healing, characterized by increased VEGF expression. This is in accordance with research conducted by Pramono WB et al., which stated that infiltration of ropivacaine increases collagen deposition in wounds. Giving ropivacaine will shorten the inflammatory phase so that the proliferation and maturation phases occur immediately and will accelerate the start of collagen synthesis.11 In another study conducted by Maharani, it was found that infiltration of Ropivacaine increased the expression (FGF) of fibroblast growth factor and affected the thickness of collagen on the third day after incision. FGF is an angiogenesis factor that plays a role in the formation of new blood vessels, granulation tissue, re-epithelialization and tissue remodeling.12

This study generally shows that Ropivacaine infiltration in the wound can affect the expression of VEGF on day 5 post-surgery. As mentioned by Sun JX et al., the use of long-acting local anesthetics will inhibit the Hypothalamus Pituitary Axis by blocking afferent stimulation, which will ultimately inhibit the occurrence of surgical stress. This is evidenced by the significant difference in the percentage of stained VEGF cells, the degree of percentage of stained VEGF cells, and the IRS score (Immunoreactivity score) between the ropivacaine and control groups on day 5 after surgery.13,15 Administration of ropivacaine infiltration can reduce surgical stress, resulting in wound healing.16,17

There were limitations in this study. First, this study was conducted on animal models as a part of preclinical testing. Therefore, future research in a clinical setting might be important to describe the effect of Ropivacaine infiltration in human wound healing. In addition, larger samples are needed in future studies to provide more accurate results.

### Table 3. The comparison between control and experimental groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Intensity</td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>2.375 (0.5)</td>
<td>2</td>
<td>0.714</td>
</tr>
<tr>
<td>Intervention</td>
<td>2.33 (0.48)</td>
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<td></td>
</tr>
<tr>
<td>Degree of percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.19 (0.4)</td>
<td>2</td>
<td>0.001*</td>
</tr>
<tr>
<td>Intervention</td>
<td>2.87 (0.64)</td>
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<td></td>
</tr>
<tr>
<td>Percentage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>41.25 (18.9)</td>
<td>40</td>
<td>0.006*</td>
</tr>
<tr>
<td>Intervention</td>
<td>60.67 (19.07)</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>IRS Score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.06 (1.61)</td>
<td>5</td>
<td>0.033*</td>
</tr>
<tr>
<td>Intervention</td>
<td>6.8 (2.57)</td>
<td>6</td>
<td></td>
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</tbody>
</table>

*Statistically significant if p-value less than 0.05

**CONCLUSION**

There is a significant difference in the percentage of stained VEGF cells, the degree of percentage of stained VEGF cells, and the IRS score (Immunoreactivity score) between the treatment and control groups on day 5 after surgery. Ropivacaine infiltration affected the expression of VEGF in postsurgical skin defects of Wistar rats.

**CONFLICTS OF INTEREST**

No competing interests were declared.

**ETHICAL CONSIDERATION**

This research has obtained ethical clearance from the Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, Airlangga University.

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**AUTHOR CONTRIBUTION**

Study conception and design conducted by Dyandri Yogi Astranto, Sahudi, and Husnul Ghaib. Dyandri Yogi Astranto is responsible for data collection, and Dyandri Yogi Astranto, Sahudi, and Husnul Ghaib are responsible for the analysis and interpretation of results. Dyandri Yogi Astranto was responsible for draft manuscript preparation. All authors reviewed the results and approved the final version of the manuscript.

**REFERENCES**


