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Expression of B-Cell Lymphoma 2 (BCL-2) Protein and Regeneration of Incision Wound Skin Surface Epithel Cells in Infiltration of Ropivacaine (Laboratory Experimental Study on Wistar Rats)

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Abstract

Background: Inadequate pain management will result in disruption in the healing process. Cell regeneration and proliferation of epithelial cells is an important part of the wound healing process. Bcl-2 is one of the regulators in apoptosis and cell proliferation. **Materials and Method**: The treatment group was given ropivacaine infiltration while the control group was not given any treatment. Each group Thirty-two male Wistar white rats were divided into 2 treatment and control groups, then given a superficial wound (incision) on the back of each rat which was divided into sub groups, each terminated on the 3rd and 7th day. Histological evaluation to assess the process of cell regeneration and proliferation as well as BCL-2 protein expression at the edges of the wound using immunohistochemistry. The data obtained will then be processed using SPSS. **Result:** The results showed that there were differences in cell regeneration and Bcl-2 expression at the wound edges between the group that was given ropivacaine infiltration and the group that was not given infiltration. **Conclusion**: Administration of ropivacaine by infiltration has the effect of increasing the proliferation of epithelial cells in the superficial wound healing of wistar rats.

Key Words: Ropivacaine, Cell Regeneration, Bcl-2

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Introduction

The skin was the body's outermost organ and serves crucial roles in maintaining life by controlling body temperature, regulating water and electrolyte balance, and protecting the body from microorganisms. However, the function was no longer adequately performed when the skin disturbed for any reason (chronic wound, burn, neoplasm, or trauma). Consequently, it was crucial to ensure its integrity as quickly as feasible (Enoch & Price, 2004).

Any wound causes a loss of skin integrity resulting in an imbalance in function, anatomy and possibly accompanied by disability or even death. Skin wound repair through a well-organized multiphase process (inflammatory stage, cell proliferation, matrix deposition to remodeling phase) between the interactions of various cell types, growth factors and cytokines that led to the wound area was aimed to seal the skin and produce tissue repair (Cohen & Mercandetti, 2016; Sunarto et al., 2019).

Multiple tissue and cell types, including inflammatory cells, fibroblasts, keratinocytes, endothelial cells, and macrophages, should work together to heal a wound. Cytokines, growth factors, and extracellular matrix (ECM) components all exert strong control over these cells. Numerous growth factors, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), transforming growth factor (TGF), and nerve growth factor (NGF), were indeed produced and released by inflammatory cells, keratinocytes, and fibroblasts in the wound and border areas (J.-C. Chen et al., 2014).

Wounds on the skin of adults could regenerated by repair or regeneration, and there were major differences between the two processes. After a loss of function, repair consists of physiological modifications that created continuity without seeking to restore the original tissue. Regeneration, on the other hand, tries to rebuild damaged tissue with identical replicas in order to restore tissue form and function. Mature mammalian skin does not spontaneously regenerate, but rather heals through scar formation (Borena et al., 2015).

However, surgical wounds cause acute pain and may result in a variety of negative outcomes due to inefficient patient care, including anxiety, hemodynamic compromise, increased morbidity, reduced physical performance and quality of life, delayed recovery, prolonged use of opioids during and after hospitalization, and increased maintenance costs (Gan, 2017).

In this instance, transmission of nociceptive impulses from the periphery to the CNS owing to pain leads in a neuroendocrine stress response, a combination of local inflammatory substances (e.g., cytokines, prostaglandins, leukotrienes, TNF-α (tumor necrosis factor-α)) and systemic neuroendocrine mediators. The dominant neuroendocrine response to pain involves interactions between hypothalamic-pituitary-adrenocortical and sympathoadrenal suprasegmental reflexes. These reflexes result in increased sympathetic tone, increased catecholamine and catabolic hormone secretion (e.g., cortisol, adrenocorticotropic hormone, antidiuretics), glucagon, aldosterone, renin, angio (Hurley et al., 2015).

The function of a cellular protein from the lymphoma family 2 B-cell (Bcl-2) was to regulate the initiation of the intrinsic (mitochondrial) and extrinsic pathways of apoptosis (Abdurrahman et al., 2022). Recent studies have shown that Bcl-2 family proteins also contributted in the regulation of other intracellular pathways that has a significant impact on cell survival, including antiapoptosis, autophagy, endoplasmic reticulum (ER) stress response, intracellular calcium dynamics, mitochondrial dynamics and energy metabolism, and cell cycle development (Hatok & Racay, 2016; Hurley et al., 2015).

The inflammatory stimulus activates intracellular signaling pathways which in turn activate the production of inflammatory mediators. Primary inflammatory stimuli, including microbial products and cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), mediate inflammation through interaction with the TLR, IL-1 receptor (IL-1R), IL-6 receptor (IL-6R), and TNF receptor (TNFR) involved in cell regeneration and cell death through the extrinsic pathway (Chen et al., 2018).

Apoptosis or programmed cell death has various purposes, including the elimination of damaged or dangerous cells, the creation of tissues during embryonic development, and the maintenance and regeneration of tissue homeostasis. The intrinsic and extrinsic pathways were two distinct classical apoptotic processes that have been identified. The activation of the pathways occurs in a variety of distinct ways due to the utilization of specific cellular signal components. The extrinsic pathway was dependent on death receptors of the TNF family such as Fas ligand (CD95) or TNFR-1 and was activated upon binding of their ligands. Upon activation, adapter proteins such as the Fas-associated death domain (FADD) or TNFR-1-associated death domain (TRADD) were recruited and activate the caspase cascade. Conversely, Intrinsic or mitochondrial apoptotic pathways were activated in response to different types of stress signals such as loss of growth factor (trophic factor) or DNA damage. It

was primarily controlled by the B-cell lymphoma protein family 2 (Bcl-2). The Bcl-2 protein was characterized by up to four homological domains Bcl-2 (BH), BH1–4, and uses pro- or antiapoptosis functions.

Members of the antiapoptotic Bcl-2 family (that is, Bcl-2, Bcl-xL, Mcl-1, and A1) protected cells from apoptotic stimuli by binding to and inactivating proapoptotic antagonists. Caspase-8 also cuts BH3-only protein BH3 - interacting-domain death agonist (Bid) protein to produce tBid fragments which then bind Bcl-2 in the outer mitochondrial membrane which leds to the formation of Bak/Bax pores and release of cytochrome c into the cytosol, and activation of the intrinsic apoptotic pathway (Lodish et al., 2016).

Following the initial hemostatic event, early wound healing was characterized by the invasion of neutrophils, macrophages, and lymphocytes, which serve as a source of inflammatory and growth-promoting cytokines. Fibroblasts migrate, proliferate, and produce extracellular matrix components, hence contributed to the creation of granulation tissue. Infiltration and proliferation of cells should be sufficient for the initial stages of normal wound healing. The early decrease in apoptosis made possible this rapid surge in cell proliferation. Subsequently, as the inflammatory response begins to subside with wound healing and scar formation, there was a substantial decrease in cell number, which has been demonstrated to be mediated by increased apoptotic cell death (Bhan et al., 2013).

Infiltration of a local anesthetic reduces the severity of pain by suppressing pain impulse transmission pathways. This results in a reduction in the secretion of the hormone glucocorticoid, which in turn eliminates one of the elements that inhibits the healing of wounds (Hancı et al., 2012). Giving a local anesthetic injection around the wound was thought to change the inflammatory response and speed up wound healing (Utariani et al., 2020).

Subcutaneous administration has shown bacteriostatic and bactericidal results (Bhaskar, 2015). Infiltration of local anesthetics could reduce the release of inflammatory mediators from neutrophils, reduce oxidant formation, has an anti-inflammatory effects by binding to G proteins (inhibiting adhesion of polymorphonuclear leukocytes, macrophages, and monocytes), increasing glutamate release, and interfering with the activity of several intracellular signaling pathways (Abrão et al., 2020).

Previous animal and human studies have shown that ropivacaine has less toxic effects than bupivacaine. From the description above, there were no studies that have directly observed the effect of local anesthetic infiltration around the wound on Bcl-2 gene expression and the regeneration process of skin epithelial cells. As such, we were encouraged to perform research on the effect of ropivacaine infiltration as a local anesthetic drug through inflammatory inhibition processes on changes in bcl-2 expression and the process of epithelial cell regeneration which plays an important role in the process of wound healing. This research was an experimental study on experimental rats wistar. Based on the background, this study aimed to analyze the effect of infiltration ropivacaine around the wound on bcl-2 expression and epithelial cell regeneration on the healing process of Wistar rat incision wounds.

Literature Review

Wound

Damage to the integrity of the skin's epithelium was what constitutes a wound, which may also be followed by disturbances to the structure and function of the normal tissue that lies beneath the damaged area. Extensive tissue damage may happen from the exact disruption of tissue with a surgeon's knife (incision) (eg major trauma, burns). Likewise, the wound may be the result of a bruise, hematoma, laceration, or abrasion (Enoch & Sumartono, 1987).

The healing of skin wounds seems to be a complex physiological process involving the collaboration of numerous cell types and biochemical mediators in a progressive and overlapping process. The healing of wound-

induced lesions begins early in the inflammatory phase. In the end, wound healing ends in repair, which consists of the replacement of specialized structures due to collagen deposition, and regeneration, which corresponds to processes of cell proliferation and differentiation via preexisting tissue cells and/or stem cells. These methods were not mutually exclusive; – in other words, regeneration and repair may occur following a skin lesion in the same tissue depending on the cell strain affected by the injury (Cristina and Gonzalez, 2016).

Apoptosis

A form of programmed cell death known as apoptosis occurs when a cell responds to various stimuli, either physiological or pathological, by undergoing a sequence of structural alterations. Apoptosis was characterized by cellular shrinkage, membrane blebbing, chromatin condensation, and nuclear disintegration. Caspases, a class of protease enzymes that could degrade the cytoskeleton and protein metabolites through proteolysis, were activated to produce this picture. These enzymes include poly (adenosine-5 disphosphate-ribose) polymerase (PARP), DNA-dependent protein kinase, lamins, proteins kinases, and actin. Extrinsic or death receptor route and extrinsic or mitochondrial pathway were the two primary apoptosis pathways (Lodish et al., 2016).

The Role of Apoptosis in Wound Healing

Neutrophils were the first cells to arrive at the trauma area because they perform an important defense function against invading organisms. Neutrophil activity was also involved in local and systemic tissue damage through the release of oxygen free radicals and proteases. Most neutrophils enter to eliminate microorganisms, undergo apoptosis, and thereafter were rapidly and efficiently phagocytosed by macrophages in a process that does not cause further inflammation (Rai et al., 2005).

Macrophages also undergo apoptosis although the mechanism was less known. Tidball and St. Pierre reported that macrophages predominate in the inflammatory response and disappear rapidly through apoptosis. However, the mechanism was suspected to be related to transforming growth factors that affect the downregulation of inflammation. In the wound remodeling phase, different cell populations need to be removed. Fibroblasts need to be downregulated along with decreasing concomitant vascularization. Preliminary studies showed that endothelial cells undergo apoptosis followed by loss of myofibroblasts. In vitro studies showed that c-myc was involved in fibroblast apoptosis in a process involving fas and fasL interactions on the cell membrane. Growth factors, especially insulin-like growth factor-1 to increase fibroblast proliferation. Conversely, another class of growth factors may be involved in decreasing fibroblast activity in wounds (Rai et al., 2005).

BCL-2

Bcl-2 was B-cell lymphoma / leukemia-2 and the second of several proteins found in lymphoma. This gene was discovered due to its participation in B-cell malignancy, which involves chromosomal translocations that activate the most of genes in non-Hodgkin's B-cell follicular lymphoma. The Bcl-2 gene has over 230 kb of DNA and consists of three exons of which exon 2 and a small part of exon 3 encode proteins. Bcl-2 encodes 2 mRNAs, namely Bcl-2α and Bcl-2β, of which only Bcl-2α seemed to be biological relevance. The Bcl-2 protein was a membrane protein that has a molecular weight of 26 kDa and was located in the cytosolic portion of the nuclear envelope, endoplasmic reticulum and outer mitochondrial and cytoplasmic membranes (Borner, 2003; Hatok & Racay, 2016).

Ropivacaine

Ropivacaine was a local anesthetic with a lengthy duration of action that was structurally similar to bupivacaine. Ropivacaine was a pure S(-) enantiomer, unlike bupivacaine which was a racemix. The S(-) enantiomer has lower toxicity and increases sensory block relative to motor block. The physicochemical properties of both enantiomers were the same, but they both has different affinities in places that might cause side

effects. The R(+) and S(-) enantiomers of local anesthetics has different affinities for sodium, potassium, and calcium ion channels. This resulted in a decrease in the toxicity of S(-) enantiomer towards the central nervous system and heart compared to R(+) enantiomer. Ropivacaine was slightly less potent than bupivacaine. Ropivacaine was suitable for use in epidural and regional anesthesia of similar duration to bupivacaine (Maheshwari & Naguib, 2015).

Ropivacaine inhibits conduction or decreases or prevents the typical transient rise in sodium permeability of excitable membranes caused by membrane depolarization. This effect was due to the interaction of ropivacaine with sodium channels (voltage-gated sodium channels). As ropivacaine acts on neurons, the threshold for electrical excitability increases, action potentials decrease, and impulse conduction slowed down. As a result, nerve conduction was obstructed (Maheshwari & Naguib, 2015).

In addition to inhibiting conduction in neurons in the peripheral nervous system, ropivacaine also affects the function of all organs where conduction or transmission of impulses occurs. Thus, the effect also occurs in the central nervous system, autonomic ganglia, neuromuscular junctions, and all muscles. The danger of side effects that occur depends on the circulating concentration of ropivacaine. In general, local anesthetics with the S-enantiomer were less toxic than the R-enantiomer (Kuthiala & Chaudhary, 2011).

Conceptual Framework

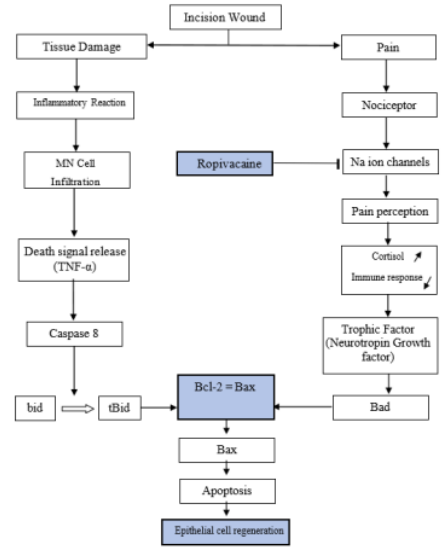


Figure 1: Conceptual framework

Information :

: Next arrow or influenced

: Variable under study

: Inhibit the process

Hypothesis:

1) There is a difference in the expression of the Bcl-2 protein which was higher in the infiltration of ropivacaine around the wound during the healing process of incisions in Wistar rats.
2) There is a difference in the higher regeneration of skin surface epithelial cells when infiltrated with ropivacaine around the wound during the healing process of incisions in Wistar rats.

Materials and Methods

Research Design

This research was an experimental study with a "randomized post test only control group design" using pure strain Wistar rats as research objects. The treatment given was infiltration of the local anesthetic ropivacaine with evaluation of Bcl-2 expression and the level of regeneration of

epithelial cells on the surface of the incision wound in the tissue. Preparations were made by HE and thricrom staining and then examined under a microscope.

Research Sample

The experimental animals were Wistar rats obtained from the Experimental Animal Care Unit (UPHP) of the Department of Pharmacology, Faculty of Medicine, Universitas Airlangga.

Inclusion criteria :

1) Pure ancestry
2) Age two to two and a half months
3) Body weight 250-300 grams.
4) No anatomical abnormalities were seen

Exclusion criteria:

1) Sick during the 7 day adaptation period.
2) Has a previous scar.
3) There were anatomical abnormalities in mice.
4) There was a 10% weight loss during the adaptation period

The size of the Wistar rat sample was determined by the formula

$$n = \frac{2\sigma^2\left(Z_{1-\alpha/2} + Z_{1-\beta}\right)^2}{(\mu_1 - \mu_2)^2}$$

N : Total number of samples
α : 0,05 → Z1/2a = 1,96
β : 0,10 → Zb = 1,28
μ1 : Average Bcl-2 expression in previous studies
μ2 : Average Bcl-2 expression in the control group
σ : SD in previous studies

Because there was no data in previous studies, based on the formula, the number of samples needed was 7,2 and rounded up to 8 per treatment group.

Research Location

The selection of experimental animals was carried out at the Department of Pharmacology, Faculty of Medicine, Universitas Airlangga. Preparations were made at the Department of Pharmacology, Faculty of Medicine, Universitas Airlangga and microscopic observations were made at the Department of Anatomical Pathology FK UNAIR/Dr. Sutomo Surabaya.

Treatment Material

The experimental animals were Wistar rats aged 2 to 2,5 months and weighing 250-300 grams. The Wistar rat was one of hundreds of strains, originating from the American continent. Widely used as experimental animals in research in the fields of medicine and veterinary medicine. The Wistar rat were obtained from the Experimental Animal Care Unit at Universitas Airlangga, Surabaya. During the experiment, the experimental animals were placed in cages and given standard feed and sufficient drinking.

a) Materials and tools for injection and wound creation (Minor surgical devices in the form of a scalpel knife, chirurgis tweezers, Tegaderm and sterile doek).

b) Materials and means for infiltration (The tools used include: 1 ml disposable syringe, 0,2% ropivacaine solution)

c) Materials and tools for making histopathological preparations (The tools and materials needed include: 10% formalin buffer, alcohol (50%, 70%, 80%, 96%, 100%), xylol, liquid paraffin (Histoplast), Van Gieson stain, and Canadian balsam).

Research procedure

1) The selection of Wistar rat was carried out at the Corba Animal Care Unit, Department of Pharmacology, Faculty of Medicine, Universitas Airlangga. The selection was carried out by taking into account the inclusion and exclusion criteria, so that 24 male rats were selected to be used in the study.
2) The next step was acclimatization to give the rats time to adapt to the research environment. Acclimatization was carried out for one week. Acclimatization was done by equalizing food, drink, and measuring the weight of the rats.
3) Wistar rat that have gone through the

acclimatization period could be used for research. These rat were then grouped using a simple random method, grouped into 4 experimental groups. Each group contained 8 rats which were divided into 4 cages with the size of each cage 30x20x7 cm.

4) A total of 32 rats were divided into 4 groups which were randomly assigned. Each consisted of 8 rat for the Control-1 and Control-2 groups (K1 and K2) with 0,9% of NaCl infiltration, then 8 rat each for the treatment group (P1 and P2) with ropivacaine infiltration 0,2 %.
5) All rats were anesthetized using ketamine-xylazine at a dose of 75-100 mg/kg + 5-10 mg/kg intraperitoneally for 10-30 minutes. Afterwards, the hair on the rat's back was shaved. A 2 cm incision was made on the rat's back.
6) K1 and K2 rat group were given infiltration of 0,9% NaCl as much as 1 ml around the wound approximately 0,5 cm around the wound, then the wound was closed with tigaderm. Furthermore, P1 and P2 rat group were given infiltration of 0,2% ropivacaine as much as 1 ml around the wound approximately 0,5 cm around the wound, then the wound was closed with tigaderm.
7) On day 3, 12 rats from groups K1 and P1 were anesthetized using ketamine. After the rats were anesthetized, a biopsy excision was performed on the scar tissue, which was sliced 3 cm square with a depth to the subcutis.
8) Histological preparations were made and microscopically assessed.
9) On the 7th day, 16 rats from groups K2 and P2 were anesthetized using ketamine. After the rats were anesthetized, a biopsy excision was performed on the scar tissue, which was sliced 3 cm square with a depth to the subcutis.
10) Histological preparations were made and microscopically assessed.

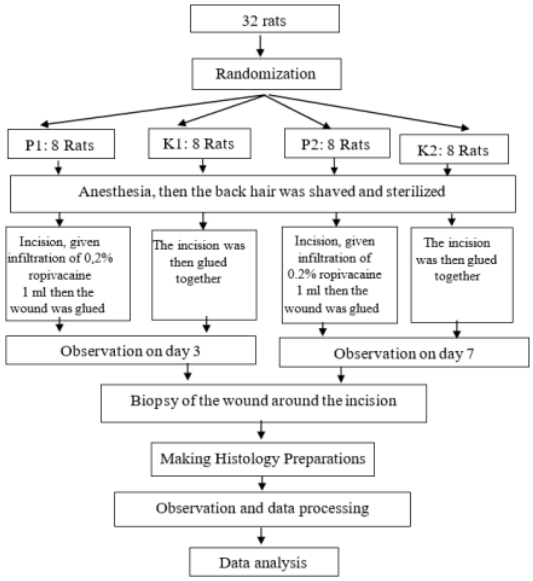


Figure 2: Operational framework

Data Analysis

The collected data were processed with the help of SPSS statistical software, then analyzed descriptive statistics in the form of percentages, graphic tables or pictures. To answer the specific objective, a parametric statistical test is used if the data curve was normally distributed, and if the data curve was skewed, a non-parametric statistical test was used.

Results and Discussion

Demographic Characteristics of Research Subjects

This study was an experimental study using pure strain Wistar rats as research objects as many as 32. The sample was then divided into 4 groups, namely 2 treatment groups were given 0,2% infiltration and examined on the 3rd day (P1) and 7th day group (P2), and the other 2 groups were given 0,9% NaCl infiltration and also examined on the 3rd day (K1) and 7th day group (K2).

Parameters observed were the expression of Bcl-2 protein in basal layer epithelial cells in the area around the wound and cell regeneration which was estimated from the proliferation affinity of epithelial cells in the area of basal epithelial cells. Observation of Bcl-2 protein expression was carried out using immunohistochemical staining examination using specific monoclonal antibodies from paraffin block preparations. Calculation of Bcl-2 protein expression using immunohistochemical staining and measurement using the Allred Scoring Guideline which was the sum of the proportion score and intensity score of epithelial cells expressing Bcl-2 protein (Maae et al., 2011).

Bcl-2 Expression and Cell Regeneration in Treatment

Tables 1 and 2 showed the characteristics of Bcl-2 expression and descriptions of cell regeneration in each study group. In this case, the highest average expression of Bcl-2 and the highest cell regeneration figure was P2 with values of 7,13 & 0,641 and 2 respectively 2,88 & 0,354. The median Bcl-2 expression and the highest cell regeneration features were P2 with values of 7,00 (6-8) and 3,00 (2-3), respectively.

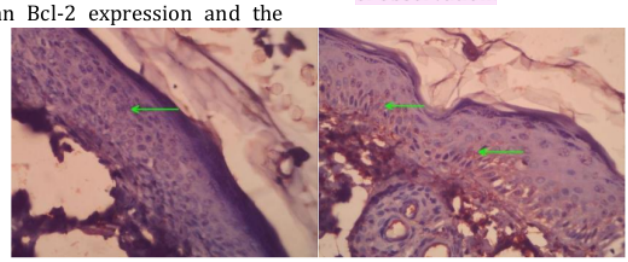
Table 1: Characteristics of Bcl-2 expression after administration of wound incisions in Wistar rats

Group	Average	Standard Deviation	Median	Minimum	Maximum
K1	4,63	0,518	5,00	4	5
P1	5,88	0,354	6,00	5	6
K2	5,75	0,463	6,00	5	6
P2	7,13	0,641	7,00	6	8
Total	5,84	1,019	6,00	4	8

Table 2: Characteristics of cell regeneration after infiltration of 0,2% ropivacaine in wound incisions of Wistar rats

Group	Average	Standard Deviation	Median	Minimum	Maximum
K1	0,75	0,463	1,00	0	1
P1	1,63	0,518	2,00	1	2
K2	1,88	0,641	2,00	1	3
P2	2,88	0,354	3,00	2	3
Total	1,78	0,906	2,00	0	3

On the 3rd day of observation (Figure 3) it was seen that Bcl-2 expression was higher in the treatment group (P1) than in the control group (K1) while on the 7th day of observation (Figure 4) Bcl-2 expression in the group (P2) was seen to be higher when compared to the control group (K2). In the treatment group Bcl-2 expression increased on the 3rd and 7th day of observation. Likewise, the control group also experienced an increase on the 3rd and 7th day of observation. The highest Bcl-2 expression was found in the P2 treatment group on the 7th day of observation.



(A) (B)

Figure 3. Expression of Bcl-2 (green arrows) on day 3 by Immunohistochemical staining (X100) in the control group (A) and the treatment group (B)

(C) (D)

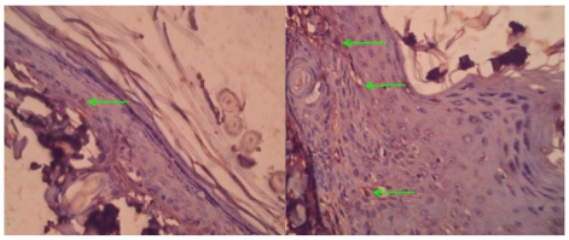


Figure 4: Bcl-2 expression (green arrows) on day 7 with Immunohistochemical staining (X100) in the control group (C) and the treatment group (D)

Effect of Ropivacaine Infiltration around the Wound on Bcl-2 Expression in the Healing Process of Incision Wounds in Wistar Rats

Table 3: The normality test results for Bcl-2 expression in each study group

Group	p-value
K1	0,0001
P1	0,0001
K2	0,0001
P2	0,037

*Shapiro-Wilk test of normality

Based on the results of the normality test, Bcl-2 expression in each study group was not normally distributed (p <0,05). The results of the different tests showed that there were significant differences between variables, except for the P1 and K2 groups which showed that there was no significant difference in the average Bcl-2 expression between the P1 and K2 groups (see Table 4).

Table 4: The results of different Bcl-2 expression tests for each study group

Bcl-2 expression different test results	Group	p-value
Research Group	K1 P1 K2 P2	0,0001*
Group K1 and P1	K1 P1	0,001*
Group K1 and K2	K1 K2	0,002*
Group K1 and P2	K1 P2	0,001*
Groups P1 and K2	P1 K2	0,535*
Group P1 and P2	P1 P2	0,001*
Group K2 and P2	K2 P2	0,001*

*Mann-Whitney test

Effect of Ropivacaine Infiltration around the wound on the description of Epithelial Cell Regeneration in the Wound Healing Process of Wistar Rats

Table 5: Normality test results depict epithelial cell regeneration

Group	p-value*
K1	0,0001
P1	0,0001
K2	0,037
P2	0,0001

*Shapiro-Wilk test of normality

Based on the results of the normality test, the description of epithelial cell regeneration in each study group was not normally distributed (p <0,05). The results of the different tests showed that there were significant differences between variables, except for groups P1 and K2, that is, there were no significant differences in the average appearance of epithelial cell regeneration between groups P1 and K2 (Table 6).

Table 6: Test results of different epithelial cell regeneration description in each research group

Test results of different epithelial cell regeneration description	Group	p-value
Research group	K1 P1 K2 P2	0,0001*
Group K1 and P1	K1 P1	0,007*
Group K1 and K2	K1 K2	0,003*
Group K1 and P2	K1 P2	0,0001*
Groups P1 and K2	P1 K2	0,424*
Group P1 and P2	P1 P2	0,001*
Group K2 and P2	K2 P2	0,004*

Discussion

Wound healing was indeed a very complex process and it was influenced by a variety of factors, including several cellular and paracellular pathways (Eming et al., 2014; Janis & Harrison, 2014; Reinke & Sorg, 2012; Rousselle et al., 2019). Wound healing has four overlapping phases, namely hemostasis, inflammation, proliferation, and remodeling (Enoch & Leaper, 2005). Any disturbance of the four phases may cause a disruption in wound healing and vice versa (Cullen et al., 2002). The cellular apoptotic mechanisms were responsible for the strict regulation and control of the processes that determine wound healing and other tissue repair processes (Rodrigues et al., 2019).

Ropivacaine, one of the long duration local anesthetics, played a role in enhancing wound healing through two mechanisms, namely anti-inflammatory and analgesic agents. Wounds in the process may cause stress and then inflammation appears, the role of neutrophils, and stimulates anti-apoptosis. Wounds also cause pain which could stimulate immune release and cortisol thereby stimulating anti-apoptosis. The role of ropivacaine in inhibiting the inflammatory process has a positive impact on wound healing, for example shortening the inflammatory process in the wound healing process (Sgonc & Gruber, 2013). Ropivacaine which acts as an analgesic might maximize wound healing through complex mechanisms, including inhibiting cortisol and the immune response (Kiecolt-Glaser et al., 1995). Previous studies using experimental models and cultured fibroblasts in wounds investigated the effect of local anesthetics, such as bupivacaine, prilocaine, and lidocaine on wound healing (Dere et al., 2009; Drucker et al., 1998; Fedder et al., 2010; Vasseur et al., 1984; Waite et al., 2010). However, the function of ropivacaine in the process of wound healing was not yet completely recognized.

The process and signaling pathways of ropivacaine in wound healing were still not fully understood, including its effects on gene expression, apoptosis, and cell regeneration. Recent studies have shown that 0,2% ropivacaine infiltration, a local anesthetic, has the best efficacy and safety (Bianconi et al., 2003; Labaille et al., 2002; Niiyama et al., 2016). In addition, local anesthetics were believed to be an easy, cost-effective, and effective modality for many surgical methods (Gupta, 2010). Hence, this study analyzed the expression of Bcl-2 protein, anti-apoptotic protein, and regeneration of the incision wound epithelium of Wistar rats after infiltration with ropivacaine. The experimental study was chosen since the pharmacokinetic and pharmacodynamic characteristics of ropivacaine are comparable in animals and humans (Arthur et al., 1988; Yi et al., 2009). Incisions on Wistar may also demonstrated the process of primary wound healing.

Effect of Ropivacaine Infiltration around the Wound on Bcl-2 Expression in the Healing Process of Incision Wounds in Wistar Rats

In this study, there were significant differences in Bcl-2 expression between variables. This shows that there was an increase in Bcl-2 expression after administration of 0,2% ropivacaine infiltration. Our data support the

concept that 0,2% ropivacaine can affect the Bcl-2 gene and confer a cell survival advantage. Following this idea, the method of wound healing could be utilized using this information. Besides, the results of this study were supported by previous studies (Özkiriş et al., 2012; Pramono et al., 2016). Initially, Bcl-2 was found in follicular lymphoma associated with a t chromosome translocation (14:18) (Adachi et al., 1990). The translocation causes overexpression of Bcl-2 which leds to inhibition of apoptosis (Hockenbery et al., 1990). On the other hand, overexpression of Bcl-2 protein has been reported in many types of human cancer, including leukemia, lymphoma, and carcinoma (Cimmino et al., 2005). Still, inhibition of apoptosis also has the potential to be involved in the wound healing process. Following injury and the onset of injury, Bcl-2 expression increases, to allow cellular proliferation to occur (Appleton et al., 1996). Expression of apoptosis-related markers such as Bcl-2 provides a novel way to regulate the pattern of apoptosis in wound healing mouse models.

Bcl-2 expression increased with increasing days, for example Bcl-2 expression was higher on day 3 compared to day 7. Even though Bcl-2 was naturally produced during the wound healing process, administration of 0,2% ropivacaine increased Bcl-2 expression. This result was supported by previous study that Bcl-2 expression in the 0,2% ropivacaine group on day 3 was the same as the group without ropivacaine on day 7. Bcl-2 increased on the second day after the appearance of the sores (Azimian et al., 2015). Some studies has shown improved regulation of Bcl-2 expression after wound healing. Studies in rats showed that immediately after injury, Bcl-2 increases to allow for cell proliferation necessary for tissue repair (Kane & Greenhalgh, 2000). Similarly, another study also showed that the low expression of Bcl-2 protein might cause deregulation of the wound healing process (Bhan et al., 2013).

The mechanism between Bcl-2 and wound healing was still not fully understood. Bcl-2 has been described as an anti-apoptotic protein. Pro-apoptotic members of the Bcl-2 family of proteins, such as Bcl-2-associated X protein (BAX) induce mitochondrial outer membrane permeabilization to cause cytochrome-c release, whereas anti-apoptotic members such as Bcl-2 act as outer membrane protectors and guard against integrity by opposing the BAX function (Furlong et al., 2013). Bcl-2 serves as a protective outer membrane and maintains its integrity (Korsmeyer et al., 2000).

Effect of Ropivacaine Infiltration Around the Wound on the Description of Epithelial Cell Regeneration in the Wound Healing Process of Wistar Rats

Wound healing studies focus on identifying molecular level target genes that might be enhanced to accelerate the wound healing process. The hedgehog signaling pathway, for example, has been used in various studies because of its role in epithelial-mesenchymal interactions in the wound healing process (Park et al., 2012). Epithelialization or the process of epithelial cell regeneration was a crucial component of wound healing which was used as a determining parameter for successful wound closure (Leoni et al., 2015). Wounds cannot be considered healed without epithelial cell regeneration. If this process does not work, the wound may develop into a chronic wound (Pastar et al., 2014). Several regulators such as growth factors and cytokines, integrins, keratin, MMPs, chemokines, and the extracellular matrix modulate epithelialization during wound closure (Pastar et al., 2014). Epithelial cells were considered to be most important in maintaining the structural integrity and function of the skin's chemical barrier (Martin, 1997). Hence, epithelial cell regeneration was a positive representation of the wound healing process.

Local anesthetics were a class of medications routinely used to alleviate post-operative pain. Long-acting local anesthetics, such as ropivacaine, were widely being used postoperatively by surgical infiltration of the incision area (Stamenkovic et al., 2021). Since the direct interaction between the local anesthetic and the surgical wound, there has been increasing interest in the possible wound healing benefits of ropivacaine. Ropivacaine was considered one of the safest long-acting local anesthetics as of its low toxicity to the

cardiovascular and central nervous systems and was most widely used in infiltration of postoperative incisions (B.-L. Li et al., 2020; K. Li et al., 2020; Wang et al., 2020). Previously, 0,2% ropivacaine increased fibroblast expression (Kurniawan et al., 2020). This was in accordance with the observations in this study, namely 0,2% ropivacaine may contribute to the healing of incision wounds in Wistar rats.

In this study, there were significant differences in the description of epithelial cell regeneration between the variables. This shows that there was an increase in the pattern of epithelial cell regeneration after administration of 0,2% ropivacaine infiltration giving an illustration of epithelial cell regeneration increasing with increasing days. In this study, it was also observed that there was no significant difference in the appearance of epithelial cell regeneration between the P1 and K2 groups. The description of epithelial cell regeneration after infiltration of 0,2% ropivacaine on day 3 was the same as that of epithelial cell regeneration without ropivacaine on day 7.

In this study, there was no significant difference in the appearance of epithelial cell regeneration between the P1 and K2 groups. The description of epithelial cell regeneration after infiltration of 0,2% ropivacaine on day 3 was the same as epithelial cell regeneration without ropivacaine on day 7. Similar research on the role of 0,2% ropivacaine in epithelial regeneration and wound healing was still limited. In addition, similar studies also stated that apoptosis decreased in the ropivacaine infiltration group when compared to the control group and decreased even more on the 7th day (Abdurrahman et al., 2022). On the other hand, previous findings also revealed that on the third day after the incision, Wistar rats that had received infiltration injections of ropivacaine around the wound experienced faster and more complete recovery from superficial wounds (Utariani et al., 2021). This might pave the way for studies on the impact of ropivacaine and other local anesthetics at concentrations of 0,2% on the healing process of wounds. To that end, our research focuses on epithelial regeneration, a critical element of the wound healing process.

The effect of local anesthetics on this process has been investigated in a number of animal studies. Although lidocaine has been reported to enhance wound re-epithelialization in rats, the effect of local anesthetics on wound healing has been a source of debate (Chvapil et al., 1979; Dere et al., 2009; Drucker et al., 1998; Madhuchandra et al., 1991). Previous studies reported that local anesthetic drugs used for infiltration anesthesia showed neutral, negative, and positive effects on wound healing (Dere et al., 2009; Nietgen et al., 1997; Vasseur et al., 1984; Waite et al., 2010). Another studies report that bupivacaine and lidocaine cause no negative or positive changes in wound healing on day 3 (Waite et al., 2010). In addition, several studies have also proven that ropivacaine exhibits anti-proliferative and anti-migratory activity in many cells (Qin et al., 2020; Yin et al., 2020; Zhang et al., 2018). The dose of ropivacaine has an impact on the role that ropivacaine plays (Scherb et al., 2009). Research in Indonesia demonstrated an increase in collagen after infiltration of 0,2% ropivacaine in rats after the 3rd and 7th day (Pramono et al., 2016). This was consistent with the theory that re-epithelialization begins after fibroblasts begin laying down proteoglycans, including new collagen and glycosaminoglycans on day 7 (Wallace et al., 2021).

However, there were a number of issues with this research. First, this research was an experimental study on experimental animals so that clinical application in humans needs to be done. Second, this study did not compare the doses and administration techniques of ropivacaine. The dosage and technique used in this study were safe and effective doses according to previous studies (Pramono et al., 2016). Third, this study did not examine the long-term effects of ropivacaine infiltration. However, according to the literature, the 3rd to 7th day was the optimal time to observe Bcl-2 expression and epithelial cell regeneration.

Conclusions

In light of the findings of this research, we concluded that there were differences in the expression of Bcl-2 in the wound healing process of the Wistar rat incisions on the 3rd and 7th day after infiltrating ropivacaine. Besides, there were differences in the description of epithelial cell regeneration in the wound healing process of Wistar rat incisions on the 3rd and 7th day after administration of ropivacaine infiltration.

In order to reap the benefits of ropivacaine infiltration on the wound healing process, the researchers suggest to consider a retrospective human study on the effect of ropivacaine infiltration in wound healing. Likewise, performing experimental animal experiments with ropivacaine at various 4 doses and procedures as well as exploring the long-term effects of ropivacaine on wound healing.

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