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FULL PAPER

The use of human dried amniotic membrane (H-DAM) as a biomaterial patch for wound healing of gastric perforation viewed from the fgf and vegf

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Gastric perforation in neonates is a medical emergency with a 75% mortality rate. Several studies have been conducted to promote tissue healing, including using various surgical procedures and materials, including H-DAM technology. To compare the expression of Fibroblast Growth Factor (FGF) and Vascular Endothelial Growth Factor (VEGF) in gastric perforation repair employing H-DAM as a patch biomaterial to that of an omental patch in New Zealand white rabbits. This experiment was carried out on a New Zealand white rabbit model divided into three groups: amniotic membrane, omental patch, and primary repair. An incision was made wide with a depth of the entire gastric wall in the gastric corpus and the perforation repair was carried out. The repair is closed with the H-DAM in the amniotic group and using the omentum in the omental group. The expression of FGF and VEGF was used to evaluate the wound healing process. VEGF and FGF expression were higher in gastric perforation models sutured with H-DAM than in models without H-DAM. There were significant differences in the average expression of VEGF and FGF. Human-dried amnion has a role in the wound-healing process in gastric perforation repair models, hence H-DAM may be the a preferred repair strategy for gastric perforation.

KEYWORDS

Gastric perforation; wound healing; dried amniotic membrane.

Introduction

Gastric perforation in newborns is a medical emergency with a 75% mortality rate [1]. The gastric perforation surgery is a primary debridement and repair procedure. Gastric perforation can be repaired using the Graham patch procedure with the omentum if the perforation is not extensive. However, because the omentum has not yet grown entirely and the size of the omentum is small in newborns, biomaterials can be used as alternative materials for flap reconstruction [2].

Much research has been conducted to increase the healing rate of post-repair tissue, including various surgical techniques and materials as additional materials in surgery. The use of H-DAM is one of the materials that has been widely researched [3]. Amnion is a suitable substance for tissue engineering since it is readily available and abundant in mesenchymal stem cells for tissue healing and

regeneration. Fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and platelet-derived growth factor (PDGF), are all present in the amniotic epithelial cell layer [4].

The aim of this study was to determine whether H-DAM is beneficial as a biopatch material for the repair of gastric perforation by evaluating wound healing based on the expression of FGF and VEGF.

Experimental

Methods

This was a true experiment with 30 New Zealand rabbits (with a average weight of 1.7 kg) with a randomized control trial study design. This study's inclusion requirements are male New Zealand White Rabbits, aged 6-9 months, weighing 2-3 kg, and being healthy and active. The exclusion requirement for this research is the rabbit did not fast for 12 hours, acted aggressively and attacked other rabbits during the 12-hour fasting period, and had surgical site infection (infection of the surgical wound during the experimental treatment, characterized by a respiratory rate > 60 breaths/minute, rectal temperature > 40 ℃, and the surgical wound is red and oozing pus). The rabbits that died before the set time were included in the dropout criteria. Laparotomy and gastric perforation were performed on the rabbits. The rabbits were then divided into three equal groups: group 1 (primary repair) received the primary repair, group 2 (omental patch) received the omental patch after the primary repair, and group 3 (H-DAM) received the H-DAM patch after the primary repair.

The rabbits were terminated on the seventh post-operative day, and the repaired gastric tissue was collected to analyze FGF and VEGF expression further. Data were collected, statistically evaluated, and compared between the control and treatment groups using Kruskal-Wallis Test, and statistically processed using the SPSS version 25.0 for Windows application.

Result

When the expression of FGF and VEGF was examined, Kruskal-Wallis test revealed a significant difference in gastric healing ($p = 0.015$ and $p = 0.005$, respectively). The mean differences between the three groups are presented in Table 1.

Post-hoc assessment was carried out to assess the comparison of FGF expression between groups, where the results revealed that there were no notable differences between the primary repair and omentum group ($p = 0.186$), and omentum and H-DAM ($p = 0.064$). A substantial difference was found in the primary repair and H-DAM group ($p = 0.007$). From the mean difference results, it was found that H-DAM had better results compared to the primary repair and omental patch group (Table 1).

Post-hoc results of VEGF expression showed that there were no notable differences between primary repair and omentum ($p = 0.053$). Significant differences were found in omentum and H-DAM ($p = 0.005$) and primary repair and H-DAM ($p = 0.034$). From the mean difference results, it was also found that H-DAM had better results compared to the primary repair and omental patch group (Table 1).

The most difficult aspect of performing research with experimental rabbits was postoperative care because so many factors influence the healing process. Some rabbits had decreased appetite following surgery, compromising the systemic healing process. Therefore, proper and enough quarantine is required before and after surgery to ensure optimal results.

TABLE 1 Mean difference and post-hoc test results of fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF)

Group	Mean	Mean difference	*P*-value
FGF			
Primary repair vs. omental patch	4.22 vs. 5.76	-1.54	0.186
Primary repair vs. H-DAM	4.22 vs. 7.57	-3.35	0.007
Omental patch vs. H-DAM	5.76 vs. 7.57	-1.81	0.064
VEGF			
Primary repair vs. omental patch	4.09 vs. 5.30	-1.21	0.053
Primary repair vs. H-DAM	4.09 vs. 7.66	-3.57	0.005
Omental patch vs. H-DAM	5.30 vs. 7.66	-2.36	0.034

Discussion

The expression of FGF and VEGF was higher in gastric perforation models sutured with H-DAM than in those without H-DAM. The average FGF and VEGF expression differed substantially between groups ($p<0.001$). The H-DAM administration improves the repair of gastric perforation and may be a therapeutic option. The amnion contains a variety of growth hormones, proteins, and stem cells that may play a role in wound healing and tissue regeneration [5]. Human amnion was selected because it is easily collected during childbirth, has minimal immunogenicity, and has been successfully utilized to treat wounds [4,6].

FGF is a group of growth factors; invertebrates have 22 FGFs which include fibroblast growth factor 1 and fibroblast growth factor 2 [7,8]. This study found significant differences in FGF levels on day 7 in each group. FGF levels in the H-DAM group were higher than in the other groups. Research by Kurniawati *et al.* found a notable difference in FGF levels on day 4 where the highest levels were obtained when using H-DAM [4]. Several FGF families have been identified to play a crucial part in the wound healing process. Sources of FGF are keratinocytes, fibroblasts, endothelial cells, smooth muscle cells, chondrocytes, and mast cells. Increased FGF-2 production occurs during the acute period and is responsible for granulation tissue formation, re-epithelialization, and tissue remodeling. *In vitro*, FGF-2 regulates the synthesis and deposition of many extracellular matrix constituents, as well as enhanced keratinocyte motility [9].

Some of the benefits of the amniotic membrane in wound healing include accelerating re-epithelialization because it stimulates migration and adhesion of epithelial cells. Growth factors that contribute to this include basic fibroblast growth factor (bFGF). The amniotic membrane secretome contains numerous substances that contribute to the regeneration capacity and activation of cell migration, including FGF [10,11]. FGF2 is a pro-angiogenic factor that induces the repair of gastric mucosal injury caused by Helicobacter pylori infection [7]. Basic fibroblast growth factor (bFGF) stimulates the healing of acetic acid-induced gastric ulcers in mice in a similar manner when given intraperitoneally or by local submucosal injection at the ulcer location, suggesting that this growth factor also accelerates mucosal repair. bFGF-mediated gastric ulcer healing involves increasing gastric blood flow toward the ulcer, suppressing gastric acid secretion, and upregulating COX-2 expression [12,13]. The use of H-DAM in a rabbit model with gastric perforation increases fibroblast formation and collagen density compared to repair using the omentum [14]. This shows that FGF application provides better gastric wound healing.

On day 7, there were substantial disparities in VEGF levels in each group, according to this study. The H-DAM group had higher VEGF levels than the other groups. VEGF expression has been reported to rise in healing skin

wounds in the first 2-5 days after injury. These findings were in line to in vitro studies in which increasing of VEGF expression on day 7 following therapy. Increasing VEGF expression until day 7 promotes the formation of blood vessels that supply nutrients to the wounded area, hence maximizing wound healing [4,15].

New blood vessels must form in order for mucosal healing to occur in the granulation tissue for wounds deeper than the epithelium layer. Growth factors influence neovascularization, just as restitution and proliferation [13]. One of the growth factors, vascular endothelial growth factor (VEGF), are very crucial for the regeneration of blood vessels through angiogenesis and vasculogenesis after mucosal injury and therefore can facilitate the healing of deep wounds [13,16]. Animal studies using topical VEGF showed increased healing of gastric ulcers and resulted in more mature blood vessels and a more complete epithelium structure in gastric ulcers caused by acetic acid in mice [17,18]. The amnion membrane has angiogenic capabilities because it can create VEGF, which is found in amniotic epithelial cells (AECs) located in the thicker basement membrane [19].

In the early stages of wound healing, H-DAM increases VEGF secretion and induces migration and proliferation of macrophages and fibroblasts. VEGF together with placental growth factor (PlGF) stimulates angiogenesis in wounds, and together with Angiopoietin1 and Angiopoietin2 regulates the stabilization and remodeling of blood vessels [20,21]. H-DAM will increase the production of growth factors and is a source of VEGF, bFGF, and other angiogenic growth factors [22,23]. The use of H-DAM has been further studied in patients with vesicovaginal fistulas where the results were that VEGF in patients using H-DAM will enhance the proliferation and migration of urothelial cells which then causes the formation of new blood vessels to be faster so that the process of transporting nutrients to the wound tissue can be achieved, maximized and helped the wound-healing process [4].

Experiment should start as a continuation to introduction on the same page. All important materials used along with their source shall be mentioned. The main methods used shall be briefly described, with references. New methods or substantially modified methods may be described in sufficient detail. The statistical method and the level of significance chosen shall be clearly stated.

Conclusion

To sum up, our study demonstrates a significant increase in both Fibroblast Growth Factor (FGF) and Vascular Endothelial Growth Factor (VEGF) expression in gastric perforation repair facilitated by Human Dried Amniotic Membrane (H-DAM) compared to repairs conducted without H-DAM. These findings underscore the pivotal role of H-DAM as a biological dressing in enhancing the healing process of gastric perforations, particularly in neonatal cases. The observed elevation in FGF and VEGF expression levels suggests that H-DAM may serve as a preferred method for repairing gastric perforations in neonates, offering promising prospects for improved clinical outcomes and reduced mortality rates. Further investigations, including clinical trials, are warranted to validate these findings and elucidate the full therapeutic potential of H-DAM in gastric perforation repair.

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Author Contribution

All authors contributed to writing the manuscript and approved the final version for publication.

Conflict of Interest

The authors declare that there is no possible conflict of interest in this study.

Ethic Approval

This study was authorized by the Research Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia (Ref. No. 2.KEH.100.06.2023).

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