The Effect of Dried Amnion Membrane Application in the Expression of Platelet Derived Growth Factors in the Healing Process of Stomach Stab Wound (Study on New Zealand Rabbit)

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Abstract

Background: Management of penetrating abdominal trauma, especially those affecting the stomach, keeps developing. Although the technology in the field of surgery is getting more advanced, the risk of complications due to leakage is still common. A lot of research is being done to improve the tissue healing process, including a variety of surgical techniques and material uses. The amniotic membrane is a material that is widely used to help stimulate the healing process. The amniotic membrane contains growth factors, one of which is platelet-derived growth factor (PDGF). PDGF is a major player in the wound healing process.

Purpose: To investigate the differences in PDGF levels in gastric rupture repair with dry amniotic membrane as a biological dressing compared to primary repair of gastric rupture without using dry amniotic membrane.

Method: This research was an experimental analytical study, using 42 samples of rabbits which were grouped into 2 groups, namely the control and treatment groups. Stab wound of 2 cm by 0.5 cm wide with a depth entire gastric wall in gastric corpus was done and repaired with 4-6 interrupted suture using 5/0 polypropylene monofilament. In the control group, the wound was only sutured and the treatment group, dry amniotic membrane was applicated before suturing. The expression of PDGF was examined from the suture tissue on day-7.

Result: Intensity score was obtained in the treatment group with the highest score of 2 with a sample of 11 (52.4%) as well as in the control group as many as 13 (61.9%) samples who had a score of 2. The extension score was obtained in the treatment group with the highest score of 1 with a total sample size of 10 (47.6%) while the control group had the highest score with a score of 0.5 as much as 14 (66.7%). After obtaining the PDGF intensity and extension values, then these two values are multiplied to get the PDGF expression. From the results of the comparison test using the Mann Whitney test, it was found that the difference in PDGF expression was significant between the treatment group and the control group with $p = 0.008$ ($p < 0.05$).

Conclusion: Applying dry amniotic membrane to the wound on the stomach will increase the expression of PDGF in the wound area.

Keywords: *dry amniotic membrane, PDGF, gastric penetrating trauma*

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Background

Management of sharp abdominal trauma, especially those affecting the gastric organs, continues to develop, in this case laparotomy and closure of defects formed

after sharp trauma to the stomach[1].

The approach to repairing injury to the stomach depends on the degree of damage to the gastric tissue and its location. Gastric injury that covers only part of the gastric wall can be performed seromuscular primary suturing with non-absorbable thread. While for gastric injury which includes the gastric mucosa, defect closure can be done by suturing the gastric mucosa with absorbable sutures followed by primary seromuscular suturing with non-absorbable sutures. In addition, a stapler can also be used to cover defects in the stomach. Even though today, technology in the field of surgery is increasingly advanced, the risk of complications due to post-suturing tissue leakage is still common. Much research has been done to improve the tissue healing process, including various surgical techniques and the use of materials (high quality surgical sutures, use of a stapler, control of sepsis with bowel preparation before surgery, use of parenteral nutrition, use of various sealants, fibrin-collagen patches, etc.), but cannot prevent the risk of these complications. Therefore, research on materials that can be applied locally to speed up the healing process or reduce the risk of leakage is of particular concern[2].

The amniotic membrane is a material that is widely used to help stimulate the healing process. The amniotic membrane has been shown to have many uses in the management of burns, oral cavity reconstruction, bladder, vagina, tympanoplasty, arthroplasty and many more[3]. The amniotic membrane is the innermost of the 3 layers that make up the placenta. The amniotic membrane is formed by 3 layers: One epithelial layer, a thick basement membrane, and a vascular mesenchymal layer[4]. The amniotic membrane contains basal membrane components, growth factors and proteinase inhibitors[5]. Research has shown that the amniotic membrane has antibacterial properties, low immunogenicity, and can aid in the epithelialization and wound healing process, inhibit inflammation and scar formation, and increase angiogenesis[4]. The amniotic membrane is also easy to obtain, easy to process and distribute. The amniotic membrane is obtained during delivery by elective cesarean section and does not need to kill human embryos for isolation, thus avoiding controversies such as the use of human cells[5].

Wound healing is a complex cellular and biochemical cascade, leading to restoration of the integrity and function of a tissue. Under normal circumstances, the wound healing process follows a predictable pattern and can be divided into phases. Several types of growth factors and cytokines are released in this process[6]. One of the growth factors that have an important role in the wound healing process is platelet-derived growth factor (PDGF). However, in conditions of sepsis, the response of platelet-derived growth factor (PDGF) to tissue damage is decreased and the addition of exogenous platelet-derived growth factor (PDGF) can improve the response to tissue damage and can reduce excessive inflammatory responses during the wound healing process.

The amniotic membrane contains growth factors including epidermal growth factors (EGF), platelet-derived growth factor (PDGF) and transforming growth factor beta. PDGF is a growth factor that first appears in the wound healing process which plays a major role in wound healing and is a major player in the wound healing process. The initial function of PDGF is to stimulate the formation and proliferation of fibroblasts. The presence of PDGF will accelerate wound healing. The next function is to induce myofibroblast phenotype. Meanwhile, EGF only functions in the reepitelialization process. Apart from the amniotic membrane, PDGF is also believed to be produced by human body tissues.[7]

There are many advantages to using the experimental rabbit animal model. Rabbits are easier to come by and easier to raise, and closer to primates phylogenetically. In addition, the cost of managing rabbits is relatively cheaper than animal models with larger size. All of these characteristics make the rabbit our experimental model of choice. Therefore, this study used a experimental animal model of male New Zealand white rabbits. Based on the above thinking, the researcher wants to conduct a study to evaluate differences in PDGF levels in t defects

Method

This research is an experimental study with a randomized control trial study design using rabbits. The experimental rabbits will be divided into two groups, each with the same number of samples. In both groups, 2 cm of gastric rupture was performed in the gastric

corpus and all-layer rupture repair was performed with 4-6 stitches one by one using 5/0 monofilament polypropylene thread. In the PA group, the anastomosis was covered with a dry amniotic membrane with a width of 2 cm x 3 cm, with the basement membrane facing the serous surface of the stomach. The amniotic membrane is fixed on the gastric serosa with 2-3 sutures. From these two groups, the specimens were sacrificed on day 7. The 5 mm rupture repair segment was cut, and fixed in 10% formaldehyde for histopathological examination.

Results

The sample of this study involved 42 experimental rabbits with injuries (rupture) 2 cm long with a width of 0.5 cm with a depth of the entire gastric wall in the gastric body and rupture repair with 4-6 stitches one by one using 5/0 monofilament polypropylene thread. . The sample was further divided into two groups, namely the sample with the primary repair group for gastric rupture (without using dry amniotic membrane) and the group using dry amniotic membrane. In the sample calculation, 21 samples were obtained for the group without using dry amniotic membrane and 21 samples for the group using dry amniotic membrane.

Table 1 – Research Subjects Characteristics

	Control Group	Intervention Group
Mean of Age (months)	9 ± 0,83	9 ± 0,92
Mean of Weight (grams)	2500 ± 183,35	2500 ± 170,71

Based on the table above, it was found that the mean age of the study sample in the group without dry amniotic membrane was 9 ± 0.83 months and the group using dry amniotic membrane was 9 ± 0.92 months and body weight in the group without using dry amniotic membrane was 2500 ± 183.35 grams and the group with using dry amniotic membrane is 2500 ± 170.71 grams. The sample characteristics in the basic data of the study showed that there were significant differences in the characteristics between the weight and age variables of the rabbits in the two sample groups where p = 0.000 for the rabbit weight variable and p = 0.021 for the rabbit age variable. This shows that the rabbits in the two groups are not normally distributed (p> 0.05, so that the data transformation is carried out and the p value is obtained <0.05. Therefore, the variables of this study are still not normally distributed and the statistical test used is the Mann-Whitney test. U test.

Rabbits were sacrificed using penobarbital was carried out on the 7th day, then the specimen was taken and fixed with formalin. Measurement of PDGF expression is performed by assessing the intensity and percentage of positive cells to evaluate immunoreactivity. The intensity of PDGF will be expressed in terms of 0 - 3 with reference to the intensity of the colored PDGF. The extensions of stained cells were expressed as grades 0 for 1 - 9%, 0.5 for 10 - 50%, and 1 for extensions over 50%. The score will be generated by multiplying the intensity value by the extension of the colored PDGF.

In this study, evaluation of the intensity, extension and expression of PDGF using scoring methods. The score with this method is semi-qualitative and the results obtained are the result of subjective observations made by the pathologist.

The intensity score was obtained in the treatment group with the highest score of 2 with a sample of 11 (52.4%) as well as in the control group as many as 13 (61.9%) samples who had a score of 2.The extension score was obtained in the treatment group with the highest score of 1 with a total sample size of 10 (47.6%) while the control group had the highest score with a score of 0.5 as much as 14 (66.7%).

After obtaining the PDGF intensity and extension values, then these two values are multiplied to get the PDGF expression. From the results of the comparison test using the Mann-Whitney test, it was found that there was a significant difference in PDGF expression between the treatment group and the control group with p = 0.008 (p <0.05). This means that statistically applying the amniotic membrane to the closure of the abdominal defect can increase PDGF expression.

Table 2 - Measurement of PDGF Intensity, Extension and Expression between Treatment and Control Groups

Measurements	Sample Groups		P
	Intervention Group	Control Group	
Intensity			
0	-	-	0.022
1	4 (19.0%)	8 (38.1%)	
2	11 (52.4%)	13 (61.9%)	
3	6 (28.6%)	0 (0%)	
Extension			
0	1 (4.8%)	4 (19.0%)	0.014
0,5	10 (47.6%)	14 (66.7%)	
1	10 (47.6%)	3 (14.3%)	
Expression			
0	1 (4.8%)	4 (19.0%)	0.008
0,5	4 (19.0%)	3 (14.3%)	
1	3 (14.3%)	12 (57.1%)	
1,5	3 (14.3%)	0 (0%)	
2	8 (38.1%)	2 (9.5%)	
3	2 (9.5%)	0 (0%)	

Based on the results of this study, it was found that almost all samples in the treatment group had a higher PDGF expression score than the control group. Figure 5.3 shows a graph of the PDGF expression in each sample from both the control and treatment groups. The highest PDGF expression score in the treatment group was 3, as many as 2 samples, and the lowest score was 0 for 1 sample. While the highest score for PDGF expression in the control group was 2 for 2 samples, and the lowest score was 0 for 4 samples.

Discussion

Gastric repair is a process that involves systemic, local, and operative factors that simultaneously influence the series of wound healing processes. Wound healing is a form of cellular response to tissue injury or injury and involves the activation of keratinocytes, fibroblasts, endothelial cells, macrophages, and platelets. This process includes regular cell migration and involvement of endothelial cells for angiogenesis (There are various growth factors and cytokines released to maintain wound healing. After homeostasis is achieved, the wound will experience 4 phases of healing, namely the inflammatory phase, epithelialization phase, proliferation phase, and maturation phase, where in the proliferation phase, fibroblasts have a major role in tissue proliferation and collagen synthesis (the main protein structure of the body)[8].

Wound healing is a complex process that begins with a disturbance in the integrity of the tissue. Several types of growth factors are found in the wound healing process which have an important role in this process. Several types of growth factors have been approved for use as treatment in humans. These growth factors are PDGF, FGF-2, IGF, and KGF[9]. PDGF is the first growth factor that appears in the wound healing process. This

shows that PDGF plays a very important role in this process. PDGF acts as a chemotactic factor. The role of PDGF here is to attract neutrophil cells, monocytes, fobroblasts, and smooth muscle cells to the wound site. In addition, PDGF also plays a role in fibroblast proliferation and production of the extracellular matrix[7].

Based on the results of this study, it was concluded that there was a significant difference in PDGF expression between the treatment group and the control group with a value of p = 0.008. This means that in the treatment group the PDGF expression is greater than the PDGF expression in the control group. Clinically, it means that the group that has greater PDGF expression has a faster recovery. This is in accordance with Werner's research saying that the PDGF level in wounds that did not heal was much less[7]. PDGF has the greatest role before day 7 of the wound healing process. PDGF plays a role in accelerating the initial inflammatory response which will speed up the wound healing process. In addition, PDGF is a major player in the wound healing process.[10]

PDGF has the greatest role before day 7 of the wound healing process. PDGF plays a role in accelerating the initial inflammatory response which will speed up the wound healing process. In addition, PDGF is a major player in the wound healing process[10]. One of the ways PDGF accelerates the wound healing period is by being an antioxidant in wound healing. In wound healing, Reactive Oxygen Species (ROS) are produced in large quantities by inflammatory cells and can cause cellular damage (called "oxidative stress") by membrane lipid peroxidation, inactivation of sulfidrile enzymes, protein cross-linking, and DNA breakdown. Detoxification of ROS can be achieved by a variety of enzymes (eg superoxide dismutase-SOD, catalase-CAT) and non-enzymatic types (eg glutathione-GSH, and antioxidant ascorbic acid-AA). However, ROS, like hydrogen peroxide (H2O2) and superoxide (O2-), can act as messengers between cells. Production of H2O2 itself is required for PDGF signal transduction. In addition, the production of ROS is important for eliminating contaminating bacteria in wound healing. Therefore, maintaining a balance between oxidants and antioxidants is very important. Because if there are too many oxidants such as ROS and are not balanced with the number of antioxidants, it can prolong the inflammatory phase and damage many cells, whereas if there are too many antioxidants such as PDGF and not balanced with the amount of oxidants (ROS), it will facilitate the infection process in wounds This is because the contaminating bacteria does not kill because there are too few oxidants.[11]

In a study conducted by Kaltalioglu et al, in 2012, it was found that NO, which has a positive effect on wound healing in low amounts and shows toxic effects in high amounts (as the main oxidant), can be suppressed in the presence of PDGF (as an antioxidant). It is known that NO is produced by several different cell types in wounds where there are three different isotypes of nitric oxide synthase. Inducible NO (iNOS) is one of these three isotypes. Fibroblasts, macrophages, keratinocytes, platelets increase the number of NO via iNOS. However, it was found in the study that NO production was significantly decreased by the presence of PDGF-AB and PDGF-BB in these cells. The production of nitrite is inhibited by PDGF-BB and causes a decrease in the formation of iNOS protein so that the production of NO can be suppressed and inflammatory events can also be suppressed.[11]

The amniotic membrane itself is known to contain a lot of PDGF, both AA, BB, AB, CC and DD PDGF. So that the use of the amniotic membrane itself is the same as applying PDGF to the wound area, because this study shows the wound area given by PDGF has a higher PDGF expression. We suspect that this amniotic membrane stimulates the expression of PDGF and several other growth factors. Rahman's research in 2013, stated that the amniotic membrane is related to interstitial collagen types I, II and elastin. In addition, the elasticity of the amnion is mainly due to type III collagen. The mechanical integrity of the amnion is maintained by interstitial collagen (types I and III) which predominate and form parallel bundles. And there are also collagen types V and VI that connect the filaments between the collagen interstitials and the epithelium. The discovery of many components of the extracellular matrix in the amniotic membrane, in our opinion, is one of the mechanisms causing the increased expression of PDGF in the group of mice that were treated with dry amniotic membrane closure. In addition to the above mechanisms, Koob et al., Found a biological component in the dry amniotic membrane

and its implications for chronic wound healing. In their research, Koob et al. find biological components such as growth factors (bFGF, EGF, GCSF, PDGF-AA, PDGF-BB, PLGF, TGF alpha and TGF beta 1), Interleukin (IL-4, IL-6, IL-8 and IL-10) and Tissue Inhibitors og metalloproteinases (TIMP-1, TIMP-2, and TIMP-3). The presence of other active ingredients besides PDGF in the amniotic membrane makes these ingredients cooperate with each other and accelerates wound healing. In his research, it was also found that in the amniotic membrane, levels of PDGF-AA were higher than PDGF-BB.[12]

Conclusion

Application of dry amniotic membrane to the wound on the stomach will increase the expression of PDGF in the wound area

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