

The Effects of Human Amniotic Membrane on Healing of Colonic Anastomosis in Dogs

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Background: Anastomotic leakage is claimed to be responsible for about one third of deaths following colon surgeries. Therefore, research on applied materials that may prevent leakage and improve healing requires more attention.

Objectives: This study was conducted to determine surgical and histological outcomes of applying human amniotic membrane (HAM) in colonic anastomosis in dogs.

Materials and Methods: Eight cross-breed male dogs were divided into two equal groups. After anesthesia and exploration, 5cm of left colon was resected, and end-to-end anastomosis was performed in a single layer. In the treatment group (B), HAM patch measuring 2×3 cm was wrapped around the anastomotic line. Mann-Whitney U test was used to compare the results in the two groups due to small sample size, and normal distribution of data was examined using the kolmogorov-simirnov test ($P = 0.03$).

Results: Modified scoring system for surgical wound healing was used to identify the grade of healing in all samples. The healing score was significantly higher in the HAM group ($P = 0.01$).

Conclusions: HAM plays a positive role in healing of colonic anastomosis, and would lead to better histological outcomes compared to simple anastomosis in dogs.

Keywords: Colon; Colorectal Surgery; Biologic Dressing; Biocompatible Materials

1. Background

Anastomotic leakage and dehiscence are frequent complications of colorectal surgery. The occurrence of anastomotic leakage varies from 2.4% to 69% (1). Several factors including surgeons' skill, and the patients' general and local conditions influence these complications. Despite overall improvements in preoperative management and surgical methods, these complications are still serious problems, which increase the morbidity and mortality rates (1-3). Anastomotic leakage is claimed to be responsible for about one third of deaths following colon surgeries. Therefore, research on applied materials that may prevent leakage and improve healing requires more attention (4). There is a growing tendency towards application of human amniotic membrane (HAM) as a biologic dressing to promote wound healing process. Anti-bacterial proper-

ties, low immunogenicity, high potency of differentiation, and easy availability are some advantages of HAM, which have encouraged researchers to examine the effectiveness of this biomaterial in surgical procedures (1, 5-9). In a previous animal study in Turkey, HAM was used to protect colon anastomosis in Wistar rats, and led to desirable outcomes (1).

2. Objectives

This study was conducted to determine surgical and histological outcomes of applying HAM in colonic anastomosis in dogs.

3. Materials and Methods

Eight cross-breed male dogs, 10-12 months of age, weigh-

Implication for health policy/practice/research/medical education:

Research on applied materials that may prevent leakage, and improve wound healing would lead to better surgical outcomes.

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ing 23-27 kg were randomly selected from 19 male dogs preserved by animal laboratory of Shiraz University of Medical Sciences in Feb 2013. All animals were initially assessed by the same veterinarian for any underlying disease. Dogs were housed in separate cages and treated with regard to the guideline instructions for care of laboratory dogs provided by Shiraz Animal Laboratory Centre in accordance with the global standards of laboratory biosafety guidelines. The study was approved by the Research and Ethics Committee of Shiraz University of Medical Sciences. The HAM used in this study, was provided by Shiraz Burn Research Center, preserved in glutaraldehyde, and then frozen in -20°C . Protocol of anesthesia and all procedures, preoperative and postoperative care, and slaughtering were identical for all cases.

Animals did not receive mechanical or chemical bowel preparation. Animals were divided into two equal groups. Group A was candidate for colon anastomosis with HAM patch, and group B including 4 dogs underwent simple one layer anastomosis. Anesthesia was induced by Intravenous Diazepam (10 mg) and Sodium Thiopental (0.5 mg) after endotracheal intubation. Then animals were maintained on controlled ventilation by halothane and 100% oxygen. Ringer's lactate was administered intravenously throughout the operation at a rate of 8 mL/kg/h. Before induction, a single dose of IV antibiotic (Ceftriaxone 1gr) was administered to the both groups.

Under general anesthesia and aseptic condition, abdomi-

nal wall was opened in supine position in anatomical layers via a midline incision. After exploration, one-third of colon from distal was identified. Then 5cm of left colon was resected, and end-to-end anastomosis was performed in a single layer with separate suture of vicryl 3/0 in the both groups. In the case group (A), HAM patch size 2×3 cm was wrapped around the anastomotic line, and fixed with vicryl 3/0 to prevent slippage. In the control group (B), no wrapping was performed. After homeostasis and abdominal irrigation with warm normal saline, abdominal wall was closed in anatomical layers. No oral feeding was given during the first day; fluid diet was started on the second day, liquid diet in the third day, and full alimentation on the 4th day post-operation. All dogs survived up to the time of slaughtering. All dogs were slaughtered by intravenous nesdonal at the end of the 4th week. Then whole colon was excised and placed in Formalin.

Multiple cross-sections were taken from the anastomosis site. They were stained with standard Hematoxylin and Eosin. The impact of HAM in repairing colon anastomosis area was considered as histological outcome. The slides were blindly studied by the same pathologist. Histo-pathological findings were evaluated for each sample according to our modified scoring system. The scoring system was based on the previous one proposed by Abramov et al. (10). The main revised factor used in this system was histological evidence of tissue necrosis (Table 1) (7).

Table 1. Wound-Healing Histological Scoring System

Score	Epithelialization	Collagenization	Inflammation	Neovascularization	Necrosis	Granulation tissue
1	none	none	severe	none	extensive	none
2	none	none	moderate	none	focal	immature
3	partial	partial	mild	< 5/HPF ^a	none	mild mature
4	complete, immature	complete, irregular	none	6-10/HPF	none	moderately mature
5	complete, mature	complete, regular	none	> 10/HPF	none	fully mature

^a Abbreviation: HPF, high power field

3.1. Statistical Analysis

SPSS for Windows version 21 statistical software (SPSS, Chicago, IL) was employed to perform the statistical analyses. Mann-Whitney U test was performed, due to small sample size, to compare the two groups and the normal distribution of data was examined by kolmogorov-simirnov test ($P = 0.03$). Two-tailed P value less than 0.05 was considered statistically significant.

4. Results

All cases of both groups survived up to the time of slaughtering. Although animals did not tolerate nasogastric tube, they experienced no problem after oral feed-

ing, and none of them developed any sign of intestinal obstruction such as vomiting.

4.1. Microscopic Evaluation

Microscopic evaluation of samples was performed by the same blinded pathologist. Modified scoring system for surgical wound healing (1) was used to identify the grade of healing in all the samples. Healing was scored according to epithelialization, collagenization, inflammation, ulcer and necrosis of the samples (Table 1). Mann-Whitney U test revealed a significant difference regarding the histological healing score of group A (mean \pm SD = 14.75 ± 0.96) and group B (mean \pm SD = 18.50 ± 0.57), $P = 0.01$ (Table 2).

Table 2. Histologic Scores of the Cases

	Dog 1	Dog 2	Dog 3	Dog 4	P value
Group A and group B					0.01
Group A (anastomosis + HAM^a)	18	18	19	19	
Group B (Simple anastomosis)	14	14	15	16	

^a Abbreviation: HAM, human amniotic membrane

5. Discussion

It is widely accepted that anastomotic leakage and dehiscence play important roles in prognosis of patients undergoing colorectal surgeries (3, 4, 11). In this study, the effectiveness of HAM as a biologic dressing was assessed in the wound healing process of colonic anastomosis and prevention of leakage and subsequent complications in dogs. Findings showed better histologic outcomes. However, in terms of surgical findings no significant difference was detected between two groups. In a previous study Uludag et al. examined the effect of HAM on colonic anastomosis in rats, and reported lower dehiscence rate, anastomotic leakage, intra-abdominal abscesses, intestinal obstruction, and adhesion formation (1). Moreover, they found less inflammation and better healing in histologic evaluation (8, 9). Application of HAM in other animal studies in different parts of gastro-intestinal tract such as duodenum and rectum led to similar results (7, 12, 13). In addition, several studies have reported accelerated wound healing process by application of HAM in burned patients (14, 15). The exact mechanism of HAM is not clear yet. However, some authors have suggested that growth factors of HAM may motivate fibroblast growth and neo-vascularization in the anastomotic site (6, 9, 16, 17). Furthermore, cell viability of HAM grafts is necessary for some characteristics such as anti-adherent properties. In a study, Polimio Di Loreto et al. applied dried and irradiated HAM as an anti-adherent layer for intraperitoneal placing of polypropylene mesh, and reported that it did not prevent adhesion formation. Therefore, it seems that existence of vital pluripotent cells is essential for some features of HAM (18, 19). On the other hand, Giuratrabocchetta et al. applied biological glues to protect colonic anastomosis in rabbits. Although more intense tissue neoformation was reported, they did not find significant difference regarding inflammation and anastomotic dehiscence for using HAM compared to sutures alone (4). At the same time, in our study according to the modified histologic scoring system, better epithelialization, and collagenization, and less inflammation and necrosis were found in microscopic evaluation of HAM group. In conclusion, HAM plays a positive role in healing of colonic anastomosis, and would lead to better histological outcomes compared to simple anastomosis in dogs. The authors believe that working on large size animals with

more similarities to human beings (regarding GI tract) would provide better context in comparison with human cases (20).

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Authors' Contribution

Neda Najibpour, revising the article, acquisition of data, final approval; Mohammad Bagher Jahantab, acquisition of data, drafting the article, final approval; Masoud Hosseinzadeh, conception and design, revising the article critically, final approval; Reza Roushanravan, acquisition of data, drafting the article, final approval; Sam Moslemi, acquisition of data, drafting the article, final approval; Salar Rahimikazerooni, analysis and interpretation of data, revising the article for intellectual content, final approval; Ali Reza Safarpour, acquisition and analysis of data, final approval, drafting the article; Leila Ghahramani, analysis and interpretation of data, revising the article for intellectual content, final approval; Seyed Vahid Hosseini, Supervisor, conception and design, revising the article critically, final approval

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