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Author(s)	El-Daharawy, Mohamed; Helal, Ibrahim
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FULL PAPER

Human amniotic membrane and vitamin E /selenium for control of postoperative adhesion in dogs

Mohamed El-Daharawy[1)] and Ibrahim Helal[1)]

1-Dept. of Surgery, Anesthesiology & Radiology -Fac. of Vet Med., Suez Canal Univ., Ismilia, Egypt. Postal code: 41522

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Abstract

This study was undertaken to compare between the human amniotic membrane (HAM) and intraperitoneal vitamin E (Vit E) and selenium in prevention of postoperative adhesions in dogs. A total of 18 apparently healthy adult Mongrel dogs were divided into three equal groups and the group (I) was treated with a sterile solution of 0.9% sodium chloride intraperitoneally as a control. Group II was treated with the HAM at jejunal enterotomy while group III was treated with Vit E and selenium administered intraperitonally. Dogs were euthanized 30 days postoperatively for histopathological examination. The results showed that both HAM and Vit E and selenium were effective in reduction of the postoperative adhesion in comparison with the group I. In terms of extent of adhesions, there was no significant difference between the HAM group and the Vit E and selenium group.

Key words: Amniotic membrane, antiadhesive, dogs, vitamin E.

Introduction

The most common cause of intra-abdominal adhesions is a history of previous abdominal surgery[16)]. Postsurgical adhesions severely affect the quality of life, causing small-bowel obstruction, difficult reoperative surgery, chronic abdominal and pelvic pain[12)]. Several treatment strategies have been evaluated to prevent or minimize the occurrence of adhesions. These include improving surgical techniques, using pharmacologic interventions targeted at the inflammatory response and fibrin deposition, and using agents that provide a physical barrier to adhesion formation[13)].

Amniotic membrane has been used in prevention of postoperative adhesions[10,19,23)]. Studies indicate that this membrane possesses antibacterial properties and low immunogenicity, can promote epithelization and wound healing, inhibit inflammation and scarring, and regulate angiogenesis[5,20,21)].

Vit E theoretically has interesting biological properties. *In vitro* studies have shown that Vit E has antioxidant, antiinflammatory, anticoagulant and antifibroblastic effects and decreases collagen

*Corresponding author: Dr. Ibrahim Elsyed Helal, Dept. of Surgery, Anesthesiology & Radiology -Fac. of Vet Med., Suez Canal Univ., Ismilia, Egypt
Phone: +0020100493837. Fax: +0020643207052. E-mail: ibrahim_surg@yahoo.com

production[14]. These properties have led to the investigation of Vit E in various studies for the prevention of adhesions[4,17].

This study aimed to compare between the effect of HAM and intraperitoneal Vit E and selenium in prevention of induced peritoneal adhesions in dogs.

Materials and methods

This study was performed at the Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Suez Canal University, Egypt and was approved by the Institutional Ethical Committee.

Animals: A total of 18 apparently healthy adult Mongrel dogs with a mean ± SD (20.9 ± 2.27) month old from both sexes and weighing 12–15 kg were used in this study. Dogs were divided into three groups each one consists of 6 individuals.

Anesthesia and preoperative technique: Dogs were fasted for 12 hs before the surgical procedure. Each dog was premedicated with intramuscular injection of chloropromazine hydrochloride in a dose rate of 1 mg/kg, 15 min prior to the induction of general anesthesia[7]. The surgical site was then clipped, shaved and disinfected with povidone iodine solution (Betadine: Povidone-Iodine U.S.P. 10%, El Nile-Co.). General anesthesia was conducted by intravenous injection of thiopental sodium 2.5% until the main reflexes were abolished. The whole animal, except the sites of operation, was draped with sterile towel. Balanced electrolyte (0.9% Sodium chloride) solution was administered (10 ml/kg/hr) during surgery.

Surgical procedures: Ten cm ventral midline incision was made and the abdominal explorations were performed to examine the viscera. The jejunum was exteriorized and 2 cm in length enterotomy incision was performed. The enterotomy incisions were closed using a Lembert's pattern in the seromuscular layer using 3–0 polyglactin 910 (Vicryl: Manufactured Johnson & Johnson Jutl). In group I, dogs were treated with a sterile solution of 0.9% sodium chloride intraperitoneally as a control. In group II, the human amniotic membrane (Biomembrane: Sterile human amnion membrane for ophthalmic use; Jensitie, A brand of Matrix Health Care S. A.E.) was applied to jejunal enterotomy incision and stabilized with three sutures 6/0 polyglactin 910 using interrupted Lembert's pattern with the basal membrane facing the surface of the jejeunal serosa (Fig. 1a). In group III, Vit E and selenium (Hipravit-Se: Vitamine E and Selenium; Laboratorios Hipra, S.A.-AVDA. LA SELVA, 135 17170 AMER (Girona) spain) were administrated in a dose (0.1 ml of Hipravit-se/kg b.w. (equivalent to 0.007 mg of selenium and 5 mg of vitamine E/kg b.w.)) intraperitonally. The laporatomy incisions were closed using polyglactin 910 No. 0 in a simple continuous pattern. Skin edges were sutured using silk No. 0 in a simple interrupted pattern.

Postoperative care: Each animal was injected postoperatively with 1 g of amoxicillin intramuscularly (I.M.) (E.Mox: Egyptian International Pharm. Industries Company, A. R. E) and dipyrone (Analgin 50%: El-Nasr Pharmaceutical Chemicals co., Egypt.) in a dose of 10 mg/kg I.M. for 3 days. After recovery, dogs were allowed for water free choice, continued to receive intravenous fluids for 48 hours after surgery, and were gradually returned to full feed over the next 48 hours. Each dog was observed till the end of the experiment.

Histopathological examination: Dogs were euthanized 30 days postoperatively using an overdose of thiopental sodium solution. The abdominal incision, peritoneal cavity, and all abdominal viscera were evaluated for adhesions and any other post surgical changes. The adhesion assessments were evaluated as the following: 0, no adhesions; 1, minimal adhesions, mainly between the omentum and the injured operating sites; 2, moderate adhesions, i.e., between the injured operating sites and a loop of the small bowel or the abdominal wall; and 3,

severe and extensive adhesions, i.e., between the injured operating sites and several loops of small bowel and the abdominal wall[25].

Tissue specimens were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with haematoxylin and eosin and Masson trichrome for histopathologic examination[3]. Inflammation, vascularization and fibrosis were graded in all samples according to pervious description[1]. The accumulation of polymorphonuclear cells and mononuclear cells reflecting inflammation was assessed. Normal cell count was graded 0, slight increase in cells 1, marked infiltration 2, and massive infiltration 3. Neovascularization was investigated by scanning of capillary vessel proliferation with prominent endothelium. In every middle power field the number of vessels was evaluated as 0 points if neovascularization was absent, 1 point for 1–2 vessels, 2 points for 3–9 vessels and 3 points for 10 or more vessels. In the assessment of fibrosis the quantitative increase of young fibroblasts and the presence of collagen were evaluated together. The scores were between 0 and 3: 0 points if fibrosis was absent, 1 point for slight, 2 point for moderate and 3 point for dense fibrosis and hyalinization.

Statistical Analysis: Comparison among the three groups was done using ANOVA by non-parametric method "Kruskal-Wallis test" followed by pair comparisons using Mann-Whitney test.

Results

Our surgical procedures were well tolerated by all animals. All laparotomy sites were intact except in two cases, one in group II and another in group III that were observed 10 days postoperatively. These dogs were excluded from analysis.

In macromorphologic evaluation, the distribution of animals according to adhesions and mean adhesion scores of the groups were presented in Table 1, respectively. The mean adhesion scores of groups II and III were significantly lower than that in group I ($p < 0.05$). In group I at 30 days postoperatively, severe adhesion was observed in one case among enterotomy site, adjacent loop of small intestine and peritoneum (Fig. 1b). Two cases showed mild adhesion between enterotomy site and mesentry while three cases in this group showed moderate adhesions between the enterotomy site and peritoneum. No adhesions were observed in two cases in group II and three cases in group III (Fig. 1c) while other cases in both groups showed mild adhesion between enterotomy site and mesentry (Fig. 1d).

Table 1. The number of dogs within the groups according to the adhesion score and mean adhesion scores (Mean ± SE).

Adhesion score	Group I	Group II	Group III
0	0	2	3
1	2	3	2
2	3	0	0
3	1	0	0
Mean ± SE	1.83 ± 0.31	0.60* ± 0.24	0.40* ± 0.24

* Significantly different ($P < 0.05$).

The results of histopathological analysis are summarized in Table 2. With respect to inflammation, neovascularization and fibrosis, the difference between group II and III was not significant. Result of group I, was significantly different from results of both group II and III. In group I, the tunica muscularis and serosa were greatly disrupted and replaced by large amount of fibrous connective tissue with severe infiltration with inflammatory cells mostly lymphocytes, macrophages and few plasma cells. A large number of newly formed blood capillaries was noticed (Fig. 2a and b). In group II and III the extent of fibrosis and inflammation were less than those of group I while neovascularization was non significant among groups (Fig. 2c and d).

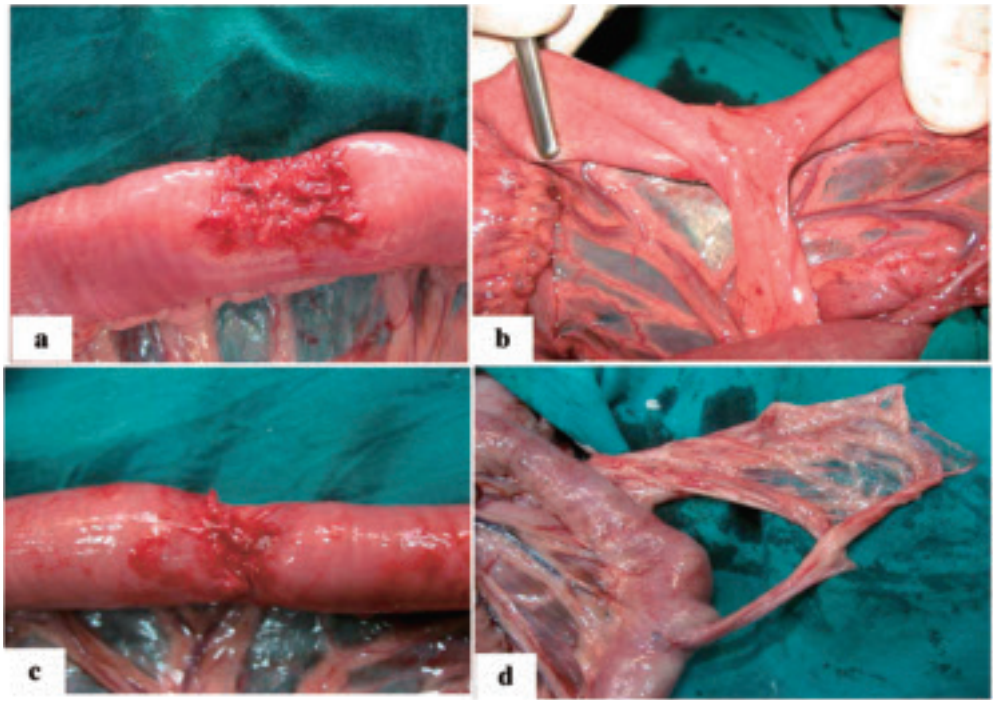

Fig. 1. (a) HAM was applied to the jejenium after closure of enterotomy incision. (b) Severe adhesion was observed in one case of group I among enterotomy site, adjacent loop of small intestine and peritoneum. (c) No adhesions were observed in one case of group II. (d) Mild adhesion between enterotomy site and mesentry in one case of group II.

Table 2. Histopathological findings in all groups (mean ± SE).

Groups	Inflammation	Neovascularization	Fibrosis
Group I	2.50 ± 0.22	2.50 ± 0.34	2.67 ± 0.21
Group II	1.20* ± 0.20	1.80 ± 0.37	0.55* ± 0.24
Group III	1.40* ± 0.24	1.60 ± 0.24	0.45* ± 0.20

* Significantly different ($P < 0.05$).

Discussion

The amniotic membrane has many characteristics which make it potentially suitable in the prevention of peritoneal adhesions. Physical barriers have been used in an attempt to prevent adhesion formation by limiting tissue opposition during the critical period of peritoneal healing. The ideal barrier should be non-reactive, absorbable and easy to use, and it should remain in the lesion site during critical stages of healing[6].

The persistence of the amniotic membrane on the damaged serosal surface without changing location played an important role in the significantly superior results[10,26]. The present study indicated that HAM was slippery and could not cover the whole lesions till complete healing of the peritoneum. Therefore, unstability of HAM might cause the increase in extent of adhesion in three cases in this group.

The slippery of the amniotic membrane was controlled in all animals with three 6/0 polyglactin sutures placed near the mesenteric aspect. Their use should be kept as few as possible, since the excessive use of sutures can trigger adhesion formation. Similarly some studies reported that the amniotic membrane had a slippery structure and recommended fixing the membrane on the injured surface with

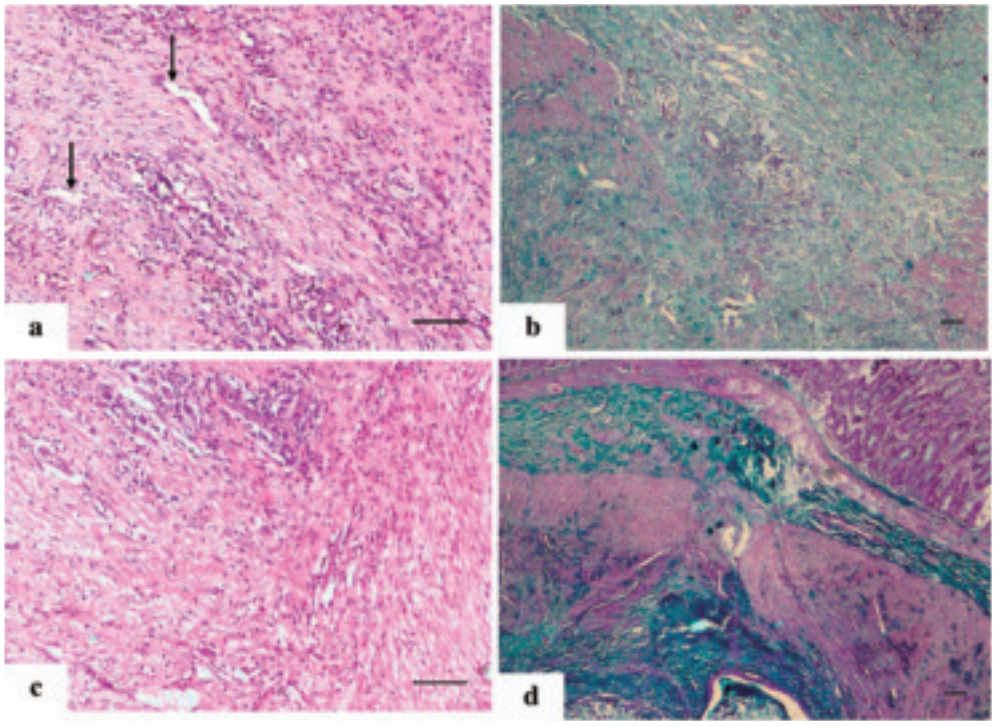

Fig. 2. (a) A photomicrograph of group I after one month showing neovascularization (arrows) and heavily mononuclear cells infiltration, H&E Stain. (b) A photomicrograph of group I after one month showing massive fibrosis replacing large area of tunica musclaris, Masson trichrome stain. (c) A photomicrograph of group III after one month showing fibrosis with mild infiltration of inflammatory cells, H&E, stain. (d) A photomicrograph of group III after one month showing mild fibrosis in serosa and partly in tunica muscularis, as well as the structure of intestinal wall is not severely impaired, Masson trichrome stain. Bars = 100 μm.

multiple 7/0 polyglactin sutures[26,27].

The results of the present study showed that both HAM and Vit E and selenium were effective in decrease of postoperative adhesion in dogs. Regarding the extent of adhesions, there was no significant difference between the group II and III.

The mean adhesion score decreased significantly in the group treated with HAM as in group I indicating that the use of HAM decreased adhesion formation in jeujenal enterotomy. Similar results were obtained in previous work indicating that HAM had an important supportive mechanical role in integrity of the anastomotic peritoneum as a biologic bandage by sticking to the serosal face, especially at the early phases of the anastomosis, in addition to its contribution to the wound healing processes[23]. On the other hand, HAM failed in prevention of adhesion in a rabbit uterine horn model[2]. The membrane used by these investigators was stored for 4–10 weeks, which might have decreased the preventive effect of the membrane on adhesion formation.

Histopathological examination of adhesions in animals which underwent HAM application revealed that, inflammation and fibrosis were significantly lower than that of the group I. These findings propose that in addition to its properties as a physical barrier, the amniotic membrane prevents adhesions by inhibition of inflammation and regulation of angiogenesis. The exact mechanism by which HAM reduced the quantity of inflammatory cells seen in the present study was blurred. This result went hand to hand with another studies[22,23].

Various mechanisms of action have been proposed to explain anti-inflammatory effects of the HAM such as inducing apoptosis and decreasing lipid peroxidation. Another possible

mechanism includes inhibiting the chemotactic activity of polymorphonuclear neutrophil and macrophages by suppressing potent inflammatory cytokines[15].

However, it was believed that placing the maternal side of the membrane against the injury is very important as it facilitates healing of the serosal injury and neovascularization contributes to the healing process as well. This result was mentioned before[26,27]. Moreover, basement membrane components contain growth factors and proteinase inhibitors[5,21]. In contrast to our belief, there were studies demonstrating that amniotic membrane transplanted onto the cornea *in vitro* and *in vivo* suppressed neovascularisation[11,18]. This condition suggested that neovascularization differed according to the location where the amniotic membrane is applied.

Vit E presents interesting biological properties and activities in prevention of intraperitoneal adhesions. There were several reports on the use of Vit E with variable success for prevention of peritoneal adhesions. The oral and intramuscular administration of Vit E in prevention of adhesions has produced conflicting results. Successful results with the use of oral vit E[9]. Oral vit E treatment decreased peritoneal adhesion incidence by 30%, and when intraperitoneal carboxymethyl cellulose was added to this treatment, the incidence decreased by 90%[8]. Oral absorption of Vit E ranged between 20 and 60%[24]. The authors therefore recommended intramuscular use of Vit E and reported successful results in their studies. Intramuscular Vit E was not sufficiently effective in prevention of adhesions and adhesions can be significantly reduced with intraperitoneal application[4]. In an interesting finding, intraperitoneal Vit E was as effective as amniotic membrane in the prevention of peritoneal adhesions[26]. To figure up, the mean adhesion score, inflammation and fibrosis, decreased significantly in the group treated with intraperitoneal Vit E and selenium compared with the group I.

The present study revealed a significant decrease in postoperative adhesion formation after using of both amniotic membrane and intraperitoneal administration of Vit E and selenium. Stabilization of HAM showed some difficulty in application procedures. On the other hand, easy administration of Vit E and selenium with an equal effect is made it preferable.

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