Short Communication

Development of an insoluble hyaluronic acid membrane as an effective material for the prevention of post-thoracotomy pleural adhesions in dogs

Akiko Uemura[1] Takashi Tanaka[1] Pinkarn Chantawong[1]

Pitipat Kitpipatkun[1] Ryou Tanaka[1*]

Abstract

A novel membrane was previously found to be effective in preventing post-thoracotomy pleural adhesions in dogs. The aim of the present study was to verify the animals' clinical condition, the biological fate of the residual membrane, the presence or absence of pleural adhesions, and any inflammatory reactions over a 10-week observation period following membrane implantation. The animals used were two male beagles. The experimental membrane, an insoluble hyaluronic acid membrane containing glycerin, was implanted under general anesthesia above the visceral pleura in the left pleural cavity of each dog after exposure to air for 30 min. A drainage tube was installed, and the incision was closed. The drainage tube was removed after confirming the absence of pleural effusion. Ten weeks after implantation of the experimental membrane, the dogs were sacrificed, and their chests were re-opened with a median sternotomy to examine adhesions. Macroscopic examination at the end of the observation period showed a total absence of pleural adhesions in both dogs, without complications of wound healing at the suture sites on the chest wall, and no findings suggested any inflammatory response. Based on these results, this experimental membrane can prevent postoperative pleural adhesions in dogs.

Keywords: dogs, insoluble hyaluronic acid, pleural adhesions, post-thoracotomy

[1]*Department of Veterinary Surgery, Faculty of Veterinary Medicine, Tokyo University of Agriculture and Technology, 3-5-8 Saiwaicho, Fuchu, Tokyo, Japan 183-8509*

[*]***Correspondence:*** *ryo@vet.ne.jp*

Introduction

Dogs have been domesticated and have eventually changed from working animals to companion animals, and nowadays being part of the family in recent years. As companion animals, dogs are now receiving preventive care and advanced treatments. Their life expectancy is reported to be 13.7 years (Inoue et al, 2015), and dogs that reach the age of 15 years old can be expected to live for a further 1.6 years (Hayashidani et al, 1988); the aging of the canine population is striking. As dogs live longer and the provision of advanced treatments in canine medicine grows, an increasing number of dogs are expected to undergo repeated surgical operations during the time they are alive.

Postoperative adhesions are an issue for patients undergoing multiple operations, and they occur with a high incidence. In human medicine, postoperative adhesions are one of the complications of abdominal surgery (ten Broek et al, 2013; Hong et al, 2015) and thoracotomy (Loop, 1984; Braxton et al., 1996). In particular, post-thoracotomy pleural adhesions have long been known to cause a range of complications at the time of re-operative thoracic surgery (Oizumi et al, 1990; Yim et al, 1996; Getman et al, 2006). To resolve this issue, several materials have been investigated to prevent this effect of postoperative pleural adhesions (Karacam et al., 2011; Takagi et al, 2013), but no product is yet available on the market.

We reported a novel membrane that was markedly effective in preventing post-thoracotomy pleural adhesions in dogs in the previous study (Uemura et al, 2017). The main ingredient of this experimental membrane was biodegradable and insoluble hyaluronic acid. We found that the experimental membrane has a very strong preventive efficacy against postoperative plural adhesions, with no worrisome clinical symptoms during the two-week postoperative observation period and no inflammatory reaction at necropsy at the end of that period. However, our previous studies (including unpublished research) also showed residual membrane in approximately 50% of the sample remaining at the time of necropsy at 2 weeks postoperative, and pleural fluid associated with its bioabsorption into the body was also observed.

The wound healing process consists of three phases (Shankar et al, 2014). The two-week post-thoracotomy period in our previous studies fell into an inflammatory phase (from wounding to around 10 days post-wounding) and a proliferation phase (from around three days to one month post-wounding), so that we were able to investigate the condition of the experimental membrane and postoperative adhesions through on these phase. However, we could not elucidate the fate of the membrane in the previous study and a role of a maturation phase (at two to three weeks post-wounding and after) on anti-adhesive effect.

Accordingly, in this study, the aim was to verify the clinical condition, the fate of the residual membrane, the presence or absence of pleural adhesions, and any inflammatory reactions at 10 weeks. This observation period covers the transition from the proliferation to the maturation phase in the wound healing process.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee of Tokyo University of Agriculture and Technology (No. 27-36). The animals used were two male beagles (TOYO beagles; Kitayama Labes, Nagano, Japan). Both animals were selected to receive the experimental membrane, and no animal was selected as a negative control, because in the previous study, which involved a 2-week observation period, showed severe adhesions between the parietal and visceral pleura at all three sites in the control group. Once stable adhesions are made, they cannot be treated naturally or medically. Thus, there was no control group in the present study for reasons of animal welfare.

The dogs were given subcutaneous cefovecin sodium (Convenia®, Zoets Japan K.K, Tokyo, Japan) at 8 mg/kg to prevent infection and subcutaneous buprenorphine (0.2 mg Buprenorphine Injection, Nissin Pharmaceutical Co., Ltd., Tokyo, Japan) at 0.02 mg/kg to palliate pain. Following these injections, pretreatment was provided with intravenous atropine sulfate and butorphanol tartrate (Vetorphale®, Meiji Seika Pharma Co., Ltd, Tokyo, Japan) at 0.2 mg/kg, and intravenous midazolam [Midazolam Sandoz (injection solution), Sandoz K.K, Tokyo, Japan] at 0.2 mg/kg. General anesthesia was then induced with intravenous propofol (Propofol Mylan, Mylan N.V., Tokyo, Japan) at 6 mg/kg. The dogs underwent tracheal intubation, after which inhalation anesthesia was maintained with isoflurane (Isoflurane for Animal Use, Intervet K.K, Osaka, Japan) at 1.4% to 2.6%. Respiration was managed with intermittent positive-pressure ventilation using the anesthesia apparatus.

The experimental membrane (10 cm × 10 cm × 0.1 cm) was an insoluble hyaluronic acid membrane containing glycerin. The membrane implantation site was the left pleural cavity for each dog. The chest was opened with an approximately 10-cm incision at the fifth intercostal space on the left side. The lung surface was exposed to air for approximately 30 min to create a mild level of invasiveness. An insoluble hyaluronic acid membrane was then implanted above the visceral pleura exposed by the incision, as an anti-adhesive agent. After implantation, a drainage tube (internal diameter: 2.5 mm, external diameter: 4.0 mm; Phycon® Tube SH No. 3, Fuji Systems, Tokyo, Japan) was installed, and the chest was closed, using conventional method.

The dogs were observed postoperatively for respiratory status, appetite, activity, surgical wound, and other signs related to general condition, and the observations were recorded. The dogs were checked for abnormalities such as pneumothorax and hydrothorax, and any drained pleural fluid was removed and its amount were recorded daily. The drainage tube was removed after confirming that no pleural fluid was being drained.

At 10 weeks after implantation of the experimental membrane, the dogs were put under

general anesthesia induced in the same manner as at implantation. Once under deep isoflurane anesthesia, each dog was exsanguinated in order to prevent errors in judgment caused by contamination of the thoracic cavity by bleeding from the sternum. After exsanguination, each dog was sacrificed by administration of an overdose of potassium chloride solution. Then, each dog's chest was re-opened with a median sternotomy.

Any adhesions found on macroscopic observation when the chest was reopened were to be dissected using Kelly forceps, Metzenbaum scissors, and a cotton swab. The severity of adhesions was evaluated based on the tenacity encountered on dissection according to the following scale: 0, no need for dissection; 1, membranous adhesion, membrane surface can easily be dissected; 2, slight adhesion, surface of adhesion can be dissected; 3, moderate adhesion, surface of adhesion is difficult to dissect; and 4, severe adhesion, surface of adhesion is impossible to dissect.

Results and Discussion

The two dogs (E1 and E2) showed no adhesion, inflammation, hemorrhage, or other lesions in the thoracic cavity at the time of membrane implantation. The experimental membrane was inserted into a pleural cavity from the approximately 10-cm thoracotomy wound, and the lung surface was exposed. This procedure was completed without any problems in either dog.

Both dogs' general condition, including activity, appetite, and respiration, was normal throughout the observation period. E1 showed no air escape from the drainage tube from postoperative day 1; however, there was accumulation of pleural fluid from postoperative day 1 to 4, but then decreased on day 4. The drainage tube was removed on postoperative day 5, when no further drained pleural fluid could be removed. E2 also showed no air escape from the drainage tube from postoperative day 1, however, pleural fluid was removed from the drainage tube once daily. Since there was still a small amount of fluid on day 4 and day 5, the drainage tube was removed on postoperative day 6, when no further pleural fluid. The pleural fluid was pale pink, and it included only a few red blood cells and white blood cells (PCV<1%) in both dogs. The condition of the surgical wound was satisfactory, and sutures were removed on postoperative day 10 in both dogs.

Macroscopic examination at the end of the observation period showed a total absence of pleural adhesions in both dogs, and the adhesion score was thus 0 in both E1 and E2. Both dogs showed normal pink coloration of the lung surface and chest wall at the thoracotomy site, without fluid in the pleural cavity or any residual experimental membrane. No discoloration, protrusions, or cicatrization was observed for any organ or tissue. There were no issues interfering healing at the suture sites on the chest wall, and no findings were suggestive of any inflammatory response (Figure 1). On the other hand, severe adhesion was observed in all of the animals in the control group (3/3) from our previous study (Uemura et al, 2017).

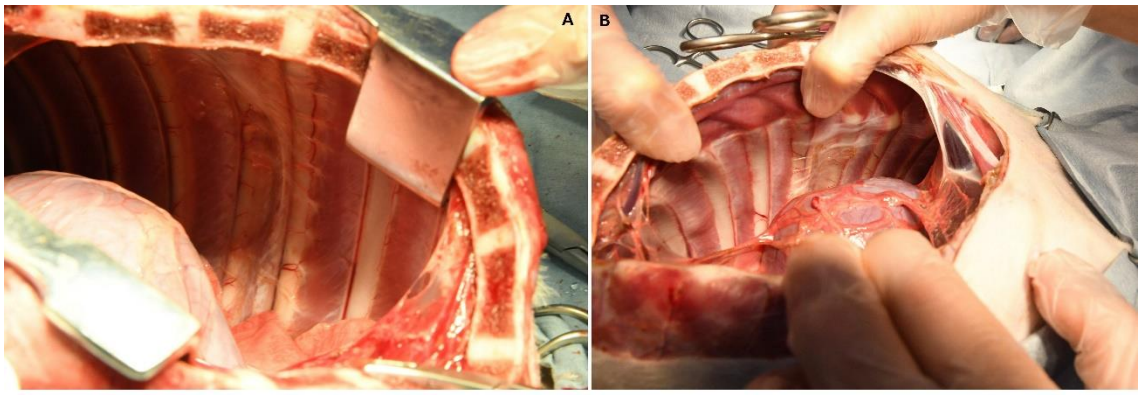

Figure 1 Macroscopic examination at the end of the observation period:
At 10 weeks after implantation of the experimental membrane, there were no complications with healing at the suture sites on the chest wall, no findings were suggestive of any inflammatory response, and the adhesion score was 0 in both E1 (Experimental Dog 1: A) and E2 (Experimental Dog 2: B).

Wound healing consists of three phases: inflammatory (from wounding to around 10 days post-wounding); proliferation (from around three days to one month post-wounding); and maturation (at two to three weeks post-wounding and after). However, the transitions between phases can be characterized by some overlap (Shankar et al, 2014). Postoperative adhesions have a different mechanism to dermal wound healing, and they are produced as a biological reaction in which fibrin bridges are formed through the inflammatory and proliferation phases of the wound-healing process (di Zerega and Campeau, 2001). The period of postoperative abdominal adhesion formation has been addressed by researchers from a range of different angles. Gonzalez-Quintero et al (2009) reported on various materials for prevention of pelvic adhesions for multiple operations (Gonzalez-Quintero and Cruz-Pachano, 2009). Sulaiman et al. (2001) reported on the mechanism of abdominal adhesions and pain sensory nerves (Sulaiman et al, 2001). Di Zerega et al. (2001) investigated postoperative adhesion formation and tissue repair at the cell level (diZerega and Campeau, 2001). Each of these reports stated that postoperative peritoneal adhesions were

formed within approximately one week. Furthermore, it is known that wound healing may be delayed depending on patient condition, foreign bodies, infection, or other factors (Shankar et al, 2014). The biodegradation of this experimental membrane dictates the period for which residual membrane is present in the pleural cavity and postoperative drainage accumulates in the pleural cavity. This could be a factor in delaying the inflammatory phase of the wound healing process. There were no reports on the timing of postoperative pleural adhesion formation. Previous research on materials for the prevention of this type of adhesion have had observation periods ranging from two weeks in mice (Izumi et al, 2012) and dogs (Noishiki and Shintani, 2010), and to 12 weeks in rats (Getman et al, 2006). These studies showed variation; however, observations were continued until complete absorbance of the bioabsorbable adhesion-preventing material in many cases.

In our previous studies, we found residual experimental membrane in around half the dogs necropsied at the end of the two-week observation period, with retention of pleural fluid in some cases. Accordingly, we had some concern over the long-term influence of the residual membrane and pleural fluid. In the present study, evaluation was performed at 10 weeks, at which was the time that the residual membrane and pleural fluid had disappeared. The two dogs showed a satisfactory general condition through the 10-week observation period, without pleural adhesion, residual membrane, or inflammatory reaction on necropsy. Based on these results, we consider that this experimental membrane effectively prevents postoperative pleural adhesions until absorption, and has a strong potential being used as a material to prevent postoperative pleural adhesions in dogs.

One concerned limitation of this study is the small number of animals. This study only involved a change in the duration of the observation period, and we had demonstrated an anti-adhesive effect for this membrane in the previous studies. Accordingly, the number of animals to be sampled was limited to two on the grounds of animal welfare. As a result, statistical analysis was not possible. However, although this study was qualitative, it demonstrated that the postoperative anti-adhesion effect shown by this experimental membrane from the inflammatory to the proliferation phase in our previous study (Uemura et al, 2017) continues into the maturation phase. Another limitation concerned is the dogs in this study were healthy animals. It is possible that animals with different health condition would have a different postoperative inflammatory reaction. We consider that future studies with larger numbers of animals are needed to investigate the anti-adhesive effect with highly invasive forms of surgery such as pericardiectomy or lung lobectomy.

In conclusion, this experimental membrane was invented to maintain its anti-adhesive effect through a 10-week observation period. This period extended beyond the inflammation and proliferation phases of wound healing, for which efficacy had previously been demonstrated, and ended after the membrane itself had disappeared. There were no signs of inflammatory reaction on macroscopic examination of the pleural cavity at the end of the observation period. The themes of future studies remain to be seen, however, we consider that this experimental membrane can be used as a material to prevent postoperative adhesions in cases of repeat thoracotomy in dogs, which is expected to be become more common, as the canine population ages.

Acknowledgements

The authors would like to thank Dainichiseika Color & Chemicals Mfg. Co., Ltd. for providing the test membrane samples used in this study. We have no sources of support in the form of grants or equipment. We have no financial or other types of conflicts of interest. This work has not yet been previously presented at any scientific meeting in whole or in part.

References

Braxton JH, Higgins RS, Schwann TA, Sanchez JA, Dewar ML, Kopf GS, Hammond GL, Letsou GV and Elefteriades JA 1996. Reoperative mitral valve surgery via right thoracotomy: decreased blood loss and improved hemodynamics. J Heart Valve Dis. 5(2): 169-173.

diZerega GS and Campeau JD 2001. Peritoneal repair and post-surgical adhesion formation. Hum Reprod Update. 7(6): 547-555.

Getman V, Devyatko E, Wolner E, Aharinejad S and Mueller MR 2006. Fleece bound sealing prevents pleural adhesions. Interact Cardiovasc Thorac Surg. 5(3): 243-246.

Gonzalez-Quintero VH and Cruz-Pachano FE 2009. Preventing adhesions in obstetric and gynecologic surgical procedures. Rev Obstet Gynecol. 2(1): 38-45.

Hayashidani H, Omi Y, Ogawa M and Fukumoto K 1988. Epidemiological Studies on the Expectation of Life for Dogs Computed from Animal Cemetery Records. Japanese journal of veterinayry science. 50(5): 1003-1008.

Hong G, Vilz TO, Kalff JC and Wehner S 2015. [Peritoneal adhesion formation]. Chirurg. 86(2): 175-180.

Inoue M, Hasegawa A, Hosoi Y and Sugiura, K 2015. A current life table and causes of death for insured dogs in Japan. Prev Vet Med. 120(2): 210-218.

Izumi Y, Takahashi Y, Kohno M and Nomori H 2012. Cross-linked poly(gamma-glutamic acid) attenuates pleural and chest wall adhesions in a mouse thoracotomy model. Eur Surg Res. 48(2): 93-98.

Karacam V, Onen A, Sanli A, Gurel D, Kargi A, Karapolat S and Ozdemir N 2011. Prevention of pleural adhesions using a membrane containing polyethylene glycol in rats. Int J Med Sci. 8(5): 380-386.

Loop FD 1984. Catastrophic hemorrhage during sternal reentry. Ann Thorac Surg. 37(4): 271-272.

Noishiki Y and Shintani N. 2010. Anti-adhesive membrane for pleural cavity. Artif Organs. 34(3): 224-229.

Oizumi H, Naruke T, Watanabe H, Sano T, Kondo H, Goya T, Tsuchiya R and Suemasu K 1990. [Completion pneumonectomy--a review of 29 cases]. Nihon Kyobu Geka Gakkai Zasshi. 38(1): 72-77.

Shankar M Ramesh B, Roopa K, Niranjan BM. 2014. Wound healing and it's importanc-a review. Der Pharmacologia Sinica. 1(1): 24-30.

Sulaiman H, Gabella G, Davis MC, Mutsaers SE, Boulos P, Laurent GJ and Herrick SE 2001. Presence and distribution of sensory nerve fibers in human peritoneal adhesions. Ann Surg. 234(2): 256-261.

Takagi K, Tsuchiya T, Araki M, Yamasaki N, Nagayasu T, Hyon SH and Nakajima N 2013. Novel biodegradable powder for preventing postoperative pleural adhesion. J Surg Res. 179(1): e13-19.

ten Broek RP, Issa Y, van Santbrink EJ, Bouvy ND, Kruitwagen RF, Jeekel J, Bakkum EA, Rovers MM and van Goor H 2013. Burden of adhesions in abdominal and pelvic surgery: systematic review and met-analysis. BMJ. 347: f5588.

Uemura A, Nakata M, Goya S, Fukayama T and Tanaka R 2017. Effective new membrane for preventing postthoracotomy pleural adhesion by surface water induction technology. PLoS One. 12(1): e0179815.

Yim AP, Liu HP, Hazelrigg SR, Izzat MB, Fung AL, Boley TM and Magee MJ 1998. Thoracoscopic operations on reoperated chests. Ann Thorac Surg. 65(2): 328-330.

บทคัดย่อ

การศึกษาประสิทธิภาพของแผ่นไฮยาลูโรนิค แอซิด ชนิดไม่สามารถละลายได้ ในการป้องกันการยึดติดของเยื่อหุ้มปอด ภายหลังศัลยกรรมเปิดช่องอกในสุนัข

อากิโกะ อัมบูร่า[1] ทาคาชิ ทานากะ[1] ปิ่นกาญจน์ จันทวงค์[1] ปิติพัฒน์ กิจพิพัฒน์กุล[1] เรียวยู ทานากะ[1]*

การเกิดภาวะยึดติดกันของเยื่อหุ้มปอด จัดว่าเป็นปัญหาที่พบได้บ่อยภายหลังการทำศัลยกรรมเปิดช่องอก ดังนั้นการศึกษานี้มีวัตถุประสงค์ เพื่อศึกษาประสิทธิภาพของแผ่นป้องกันการยึดติดของเยื่อหุ้มปอด ภายหลังการทำศัลยกรรมเปิดช่องอกในสุนัขพันธุ์ปีเกิ้ลเพศผู้ จำนวน 2 ตัว โดยประเมินจากสภาพร่างกายสัตว์ กระบวนการทางชีววิทยา การอักเสบ และติดตามว่ามีการยึดติดของเยื่อหุ้มปอดหรือไม่ ตลอดระยะเวลา 10 สัปดาห์หลังการผ่าตัด การทดลองทำโดยผ่าเปิดช่องอกแล้วปล่อยให้สัมผัสอากาศนาน 30 นาที เพื่อโน้มนำให้เกิดการอักเสบ จากนั้นฝังแผ่นไฮยาลูโรนิค แอซิด ที่ประกอบด้วยสารกลีเซอรีน ในตำแหน่งระหว่างเยื่อหุ้มปอดและผนังช่องอกด้านซ้าย สอดท่อระบายอากาศและของเหลวออกจากช่องอกก่อนทำการเย็บปิด โดยท่อระบายจะสามารถเอาออกได้หลังจากไม่พบของเหลวในช่องอก หลังจากผ่าตัด 10 สัปดาห์ทำการเปิดช่องอกเพื่อเก็บแผ่นป้องกันการยึดติด และเนื้อเยื่อโดยรอบมาวิเคราะห์ จากผลการทดลองพบว่า ไม่มีการยึดติดของเยื่อหุ้มปอดและช่องอก ไม่พบการสร้างเนื้อเยื่อทดแทนหรือการหายของบาดแผลในตำแหน่งที่เย็บแผ่นกับผนังช่องอก และไม่พบการอักเสบในสุนัขทั้ง 2 ตัว จากผลการทดลองนี้จึงสรุปได้ว่า แผ่นไฮยาลูโรนิค แอซิด ชนิดนี้สามารถป้องกันการเกิดการยึดติดของเยื่อหุ้มปอดได้จากการทำศัลยกรรมเปิดช่องอกได้

คำสำคัญ: สุนัข แผ่นไฮยาลูโรนิค แอซิดชนิดไม่สามารถละลายได้ ภาวะยึดติดกันของเยื่อหุ้มปอด ภายหลังศัลยกรรมเปิดช่องอก

[1]*ภาควิชาศัลยศาสตร์ คณะสัตวแพทยศาสตร์ Tokyo University of Agriculture and Technology 3-5-8 ไซไวโช, ฟุชู, โตเกียว, ญี่ปุ่น 183-8509*

***ผู้รับผิดชอบบทความ E-mail:** ryo@vet.ne.jp*