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KLİNİK ÇALIŞMA
RESEARCH ARTICLE

The effect of sodium hyaluronate-carboxymethyl cellulose membrane in the prevention of parenchymal air leaks: an experimental and manometric study in rats

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SUMMARY

The effect of sodium hyaluronate-carboxymethyl cellulose membrane in the prevention of parenchymal air leaks: an experimental and manometric study in rats

Introduction: *We aimed to examine effectiveness of sodium hyaluronate-carboxymethly cellulose (NaH/CMC) for sealing pulmonary air leaks during postoperative period.*

Materials and Methods: *The study was conducted in 16 male Sprague-Dawley rats. A linear insicion (length= 0.2 cm, depth= 0.1 cm) to the lung parenchyma on the inflated by a cutter was made. The animals were randomly divided; the control group (n= 8) and NaH/CMC-treated group (the study group, n= 8). Control group was left for physiologic healing while a NaH/CMC membrane was applied over the the incisional area in the study group. Then the pressure point where the air leakage observed was noted.*

Results: *No polymorphonucleer leucocytes (PMNL) infiltration was detected in control group, whereas PMNL infiltration was 0.38 ± 0.5 cell per 100 high field in study group (p= 0.234). The degree of macrophage, lymphocyte infiltration and the mean fibroblast count were found to be higher in study group compared with control group (p= 0.007, p= 0.02, p= 0.05, respectively). The mean pressure value for air leak to occur in the control group was 43.50 ± 9.55 mmHg whereas it was 73.75 ± 16.68 mmHg in the study group (p< 0.001).*

Conclusion: *The data revealed that bioabsorbable NaH/CMC membrane accelerates healing with preserving the expansile character of lung parenchyma even in high ventilation pressures. However, further studies are required to assess the prevent impact of the pulmonary air-leak for NaH/CMC membrane.*

Key words: *Experimental thoracic surgery; pulmonary air leak; sodium hyaluronate-carboxymethyl cellulose; healing*

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ÖZET

Sodyum hiyalüronat-karboksimetil selüloz membranın parankim hava kaçaklarını önleme etkisi: sıçanlarda deneysel ve manometrik bir çalışma

Giriş: *Postoperatif dönemde pulmoner hava kaçaklarının engellenmesi için sodyum hiyalüronat-karboksimetil selüloz (NaH/CMC) membranın etkinliğini araştırmayı amaçladık.*

Materyal ve Metod: *Çalışma, 16 erkek Sprague-Dawley sıçanında yürütüldü. Şişirilmiş olan akciğer parankimine doğrusal bir kesi (uzunluk= 0.2 cm, derinlik= 0.1 cm) yapıldı. Hayvanlar rastgele iki gruba ayrıldı; kontrol grubu (n= 8) ve NaH/CMC ile tedavi edilen grup (çalışma grubu, n= 8). Kontrol grubu fizyolojik iyileşmeye bırakılırken, çalışma grubunda insizyonel alana NaH/CMC membran uygulandı. Sonra hava kaçağının gözlemlendiği basınç noktası kaydedildi.*

Bulgular: *Kontrol grubunda polimorfonükleer lökosit (PMNL) infiltrasyonu saptanmazken, çalışma grubunda PMNL infiltrasyonu 100 büyüme sahası için 0.38 ± 0.5 hücre idi (p= 0.234). Çalışma grubunda makrofaj, lenfosit infiltrasyonu ve ortalama fibroblast sayısı kontrol grubuna göre daha yüksek bulundu (sırasıyla p= 0.007, p= 0.02, p= 0.05). Kontrol grubunda hava kaçağı oluşması için ortalama basınç değeri 43.50 ± 9.55 mmHg iken, çalışma grubunda 73.75 ± 16.68 mmHg idi (p< 0.001).*

Sonuç: *Veriler, biyolojik olarak emilebilir olan NaH/CMC membranın, yüksek havalandırma basınçlarında bile akciğer parankiminin genişleyebilme özelliğini koruyarak iyileşmeyi hızlandırdığını ortaya koydu. Ancak NaH/CMC membranın akciğer hava kaçağının önleme etkisini değerlendirmek için daha ileri çalışmalara gereksinim vardır.*

Anahtar kelimeler: *Deneysel göğüs cerrahisi; pulmoner hava kaçağı; sodyum hiyalüronat-karboksimetil selüloz; iyileşme*

INTRODUCTION

Pleuroparanchymal prolonged air leaks (PALs) constitute one of the main surgical complications after thoracic surgery (1). Despite routine use of sutures and stapling devices, PAL remains a significant problem in the daily practice of thoracic surgery. Its incidence has been reported in up to 50% of patients rendering it one of the most frequent postoperative complication (2). Also, PALs with an incidence of 18.2% was documented as the second most common reason for early re-operation (3). PALs can result in prolonged hospital stay and complications, such as empyema, in patients undergoing lung resection (4). Brunelli et al. have reported a significantly increased rate of empyema in patients with air leaks lasting more than seven days as compared to patients with lesser air leaks (8.2% to 10.4% versus 0% to 1.1%) (5). Therefore, the peroperative prevention of air leaks is quite important and several experimental and clinic studies were accomplished.

Different sealing materials have been studied for preventing air leaks after lung resections, and each of these different products has its specific properties and indication fields (2). These include fibrin glues, albumin based sealant, dextran based sealant, cyanoacrylate, gelatin-resorcinol cross-linked with formaldehyde (GRF), GRF with gluteraldehyde, collagen, gelatin-based tissue adhesives, flexible synthetic sealants based on polyurethane, polyethylene glycol, polyester, and naturally occurring or composite sealants made of proteins or polysaccharides (6,7).

Sodium hyaluronate-carboxymethly cellulose (NaH/CMC) is a bioabsorbable membrane which is based on a chemically modified form of hyaluronic acid which is a natural glycoaminoglycan and of carboxymethyl cellulose that's a derivative of cellulose. Some clinical studies have demonstrated that NaH/CMC significantly decreases the incidence and severity of adhesion formation, although there was no clinical or experimental study of specific to the prevention of paranchymal air leak in the literature (8,9). In the present experimental study, we aimed to analyse the effectiveness of NaH/CMC for sealing pulmonary air leaks during postoperative period.

MATERIALS and METHODS

Ethical Approval

The study was conducted after approval by the Marmara University School of Medicine Experimental and Investigative Animal Laboratuary (Laboratory Ethic Committe Certification No: 7.3.02).

Animals

Sixteen sexually mature Spraque Dawley rats were used. They weighed 250-300 g. Animals were kept under standard laboratory conditions at room temperature with a relative humidity of 50%-70% and a day cycle of 14 hours light and 10 hours dark with free access to food and water ad libitum. Animals were

treated in accordance with the standards of National Institutes of Health as described in the Guide for Care and use of Laboratory Animals.

Surgical Procedure

After intraperitoneal ketamine (100 mg/kg IM of Ketalar; Eczacibasi, Istanbul, Turkey) and chlorpromazine (0.75 mg/kg IM of Largactil; E.R.P. Istanbul, Turkey) anestesia, perioperative antibiotics (amphicillin 0.1 mg/kg IM) were initiated at induction. Lactated Ringer's solution (50 mg/mL) was given at 22 mg/kg/hour during the surgical procedure.

Cervical region was extanded and cleaned by poviiodine-iodine solution. The upper trachea was insiced, the muscular plane covering the trachea was reached and explored. After the longitudinal insicion the rat was entubated with a pediatric feeding tube. The distal end of the endotracheal tube was connected to a three lined vascular access with one end connected to the manometer and the other end to the ventilator. The animal ventilator of Rodent 425 was used with the permission of Marmara University Physiology Laboratory. The manometric system was designed as the connection of the manual pressure cuff with a T-circuit to the three way vascular line access.

After the observation of the rat respiration, lateral left thoracotomy insicion to the five intercostal space was applied and an the costal space was retracted by an endoretractor.

A linear insicion to the lung parenchyma of left upper zone with a length of 0.2 cm and a depth of 0 .1 cm by a cutter was made.

The animals were randomly divided to either the control group (n= 8) and NaH/CMC-treated group (the study group, n= 8). After the surgical insicion, control group was left for physiologic healing (no suture or staple closure) and no material was applied. After the lung parenchyma was insufflated with the manometer showing the pressures and the pressure point where the air leakage observed was noted. The lung parenchyma was insufflated again and observed whether air leak contiuned at the normal lung ventilation pressures. A modified chest tube drainage system was applied before closure and lung parenchyma was insufflated, thoracotomy was closed and chest tube was taken off when the lung was fully expanded after no air leak was observed at the rat breathing spontaneously.

After the surgical insicion, a 1 x 1.5 cm NaH-CMC membrane was applied over the surface. With the help of manual pressure system, the pressure points of air leak was noted. The modified chest tube drainage system was replaced as the same in the control group and thoracotomy was closed. All the rats were extubated when their spontaneous breathing was recovered.

Ventilatory settings during surgery were same for the groups.

Termination of the Experiment

The experiment was terminated after 7^{th} day. After anesthesia and ventilation, thoracotomy was reopened with the manometric system set. For each group maximal pressures were applied and the pressures which result air leaks from parenchymal surface were recorded by one of us blindly (A.S.).

Thereafter, left pneumonectomy was performed and the parenchymal tissue was evaluated for histopathologic examination.

Histopathological Evaluation

Histopathologic examination was performed by a board-certified pathologist blindly for the inflamatory reaction and the healing process. The parameters evaluated were presence of polymorhphonucleer leucocyte (PMNL), macrophage, lymphocyte, fibroblasts, edema, neovascularisation, collagenisation, and foreing body reaction. Histologic classification and scoring was reported according to adhesion formation model in rats which was described by Milligan and Raftery (10,11).

Statistical Analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS) for Windows (version 15.0; SPSS Inc., Chicago, IL). The mean and the standard deviation of all values were measured. Mann-Withney-U test was used for statistical analysis (Fisher's exact test was used if the expected cell count in one or more of the cells was less than five). A value of $p< 0.05$ was accepted as statistically significant.

RESULTS

All animals tolerated surgical procedure. All the rats survived uneventfully until being reopened without any postoperative complications. The comparison of two groups in for histopathological evaluation are

Table 1. Histologic scoring of lung parenchyma

	Group I (control group)		Group II (study group)		
	Mean	**SD**	**Mean**	**SD**	**P**
Total	1.00	2.14	**4.25**	**2.05**	0.02*
PMNL	0.00	0.00	0.38	0.52	0.234
Macrophages	0.13	0.35	**1.00**	**0.53**	0.007*
Lymphocytes	0.25	0.46	**1.13**	**0.64**	0.02*
Fibroblast	0.38	0.74	**1.13**	**0.64**	0.05*
Edema	0.13	0.35	0.38	0.52	0.442
Neovascularization	0.00	0.00	0.13	0.35	0.721
Collagenisation	0.30	0.48	0.20	0.42	0.739
Foreign body reaction	0.70	0.82	0.30	0.48	0.353

* These differences were statistically significant.
SD: Standard deviation, PMNL: Polymorphonucleer leucocytes.

Table 2. Pressure differences for air leak between the two groups

	Group I (Mean ± SD)	Group II (Mean ± SD)	p
Pressure pre-treatment[a]	24.75 ± 4.65	26.63 ± 4.98	0.574
Pressure post-treatment[b]	43.5 ± 9.55	73.75 ± 16.68	< 0.001

[a] After the surgical insicion, the lung parencyma was insufflated with the manometer showing the pressures and the pressure point where the air leakage observed was noted.
[b] After anesthesia and ventilation, thoracotomy was reopened with the manometric system set. For each group maximal pressures were applied and the pressures which result air leaks from parenchymal surface were recorded after 7th day.

shown in Table 1. No PMNL infiltration was detected in control group, whereas PMNL infiltration was 0.38 ± 0.5 cell per 100 high field in the study group (p= 0.234). The degree of macrophage, lymphocyte infiltration and the mean fibroblast count were found to be higher in the study group compared with the control group, and these differences were statistically significant (p= 0.007, p= 0.02, p= 0.05, respectively). Neovascularisation was not observed in control group, whereas it was 0.13 ± 0.35 cell per 100 high field in the study group (p= 0.721). Furthermore, edema, collagenisation and foreign body reaction were similar between the two groups (p= 0.442, p= 0.739 and p= 0.353, respectively).

In the first step of the operation the mean pressure of air needed to inflate the lungs are noted for each animal. The mean pressure when the air leakage observed was similar between the two groups (24.75 ± 4.65 mmHg for the control group, 26.63 ± 4.98 mmHg for the study group, p= 0.574).

After the surgical intervention control group was left for physiologic healing and the study group parenchyma covered by NaH/CMC membrane for healing. At the 7th day the mean pressures needed to maintain an air leak was calculated when the animal was on the ventilator. The comparison of two groups in the mean pressures of air leaks are shown in Table 2. The mean pressure value for air leak to occur in the control group was 43.50 ± 9.55 mmHg whereas it was 73.75 ± 16.68 mmHg in the study group (p< 0.001).

DISCUSSION

In thoracic surgical resections, lung parenchymal tissue is sealed surgically via sutures, staples, or surgical meshes. Despite their common use in the clinic, these mechanical methods are inevitably associated with lung tissue damage caused by deep piercing, ischemia, and prolonged air leaks, which represent the most common complications after (7,12). In the literature the incidence decreases depending on time after lung resection with the rate in 26% to 48% of patients

on postoperative day 1 (POD1), 22% to 24% on POD2 and still 8% on POD4 (13-17). Particularly, it could lead to prolonged chest tube stay that would increase the risk of developing infections and bronhcopleural fistula in the patients, and consequently, a longer hospital stay (18).

Yet, many clinical and experimental studies analyzed the methods of intraoperative control mechanism of PALs. Peroperative methods such as suturing, electrocautery, laser cautery, stapler suturing still ended with air leak from the corrupted surface. Then the need of PTFE grafting or bovine pericardial flapping was considered but foreing body reactions and increased costs revived the use of fibrin glue materials. Thereafter bioabsorbable or absorbable synthetic glues are used in the parenchymal air leaks (19-21).

Although several tissue adhesives are commercially available, none of them are ideal surgical sealants for repairing elastic and soft tissues such as wounded lungs, heart, and blood vessels and it is extremely challenging to achieve significant adhesion to soft tissues while minimizing toxicity, tissue damage and other side effects of the sealing materials (7). Several experimental previous studies documenting the effectiveness of absorbable materials for sealing parenchymal air leaks are carried out (20-22). In a Cochrane systematic review surgical sealants were found to reduce postoperative air leaks and time to chest tube removal but a reduction in length of hospital stay could not always be demonstrated and the researchers recomended that more and larger randomized controlled clinical trials are needed (23).

Our study analyzed the use of bioabsorbable NaH/CMC material which is routinely used in abdominal gynecologic and cardiovascular operations. There was no clinical or experimental study of specific to the prevention of PALs in the literature. We experimentally observed its effect on visseral pleura in the intrathoracic setting. This was a manometric measurement which showed a significant increased difference in air leak pressure on the NaH/CMC group. The material had covered the parenchymal surface providing a physiologic surface tolerating the shear force of air coming from the broncho-alveolar system. The mean macrophage, lypmhocyte and fibroblast score was higher in the study group which represents the healing period of wound which is covering the rough surface immediately.

When the inflammatory process of wound healing is analyzed, there was no difference between presence of polymorphonuclear leuocytes, edema, neovascularization, collegenization and foreing body reaction between the two groups. The replaced NaH/CMC material had induced no additive foreign body reaction deterioating the process of wound healing.

We analyzed the mechanism of NaH/CMC membrane to improve outcome and documented that this bioabsorbable material is capable of sealing wounds in lung parenchyma at high pressures without disturbing the healing.

There is little experience detailing the amount of positive airway pressure fibrin sealants withstands (20,22). We experimentally evaluated the sealing effect of the bioabsorbable material against alveolar air leakage with our results impeding this positive pressure air. The parenchymal air leak was observed only when two fold increase in pressure was applied to the parenchyma designating the effectiveness of NaH/CMC.

The advantage of Na/CMC besides the other preventive materials is that it's inexpensive and has no risk of viral transmission. In a study of Wong and Goldstraw, fibrin glue was reported to be expensive and had an viral transmission effect (24).

In conlusion, we found NaH/CMC to be effective for reducing PALs. It did not interfere with the healing process in the lung injury. Good tolerance of this material was evidenced by the absence of inflamatory reaction. Further studies investigating the mechanism by which NaH/CMC prevent prolonged air leaks, its optimal dose and method of application, and documentation of their safe use in humans are warrented.

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