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Non-steroid anti-inflammatory drugs reduce the efficacy of autologous blood pleurodesis

Non-steroid anti-enflamatuvar ilaçlar otolog kan plöredezinin etkinliğini azaltır

Serhat Yalçınkaya[1], Ulviye Yalçınkaya[2]

Institution where the research was done:
Dumlupınar University Medical Faculty, Kütahya, Turkey

Author Affiliations:
[1]Department of Thoracic Surgery, Kütahya Health Sciences University, Medical Faculty, Kütahya, Turkey
[2]Department of Surgical Pathology, Uludağ University, Medical Faculty, Bursa, Turkey

ABSTRACT

Background: This study aims to perform autologous blood pleurodesis in an animal model and investigate the effects of paracetamol and diclofenac on autologous blood pleurodesis.

Methods: We divided 42 female Wistar albino rats (aged three months; average weight 275±25 g) into three major groups of 14. Each major group was further divided into two subgroups of seven rats to be sacrificed at seven days for early changes and 21 days for late changes. We performed autologous blood pleurodesis in all rats at a dose of 3 mL/kg. Group C (control group) was administered saline, group P was administered paracetamol, and group D was administered diclofenac for the postoperative five consecutive days as a single dose intraperitoneally. We sacrificed the rats at the designated dates and removed the thoracic cages *en bloc*.

Results: According to macroscopical and microscopical evaluation of the specimens, paracetamol led to a similar degree of adhesions with saline, whereas diclofenac significantly reduced the intensity of the desired adhesions between the two pleural sheets (p=0.05).

Conclusion: Using anti-inflammatory analgesics following autologous blood pleurodesis may lead to unsuccessful outcome of the procedure.

Keywords: Autologous blood, diclofenac, paracetamol, pleurodesis, rat.

ÖZ

Amaç: Bu çalışmada bir hayvan modelinde otolog kan plöredezi uygulandı ve parasetamol ve diklofenakın otolog kan plöredezi üzerindeki etkileri araştırıldı.

Çalışma planı: Kırk iki dişi Wistar albino sıçanı (üç aylık; ort. ağırlık 275±25 g) 14 sıçanlık üç ana gruba ayrıldı. Her ana grup erken değişiklikler için yedinci günde, geç değişiklikler için 21. günde sakrifiye edilecek şekilde yeniden yedi sıçanlık iki alt gruba ayrıldı. Tüm sıçanlara 3 mL/kg dozunda otolog kan plöredezi uygulandı. Ameliyat sonrası beş ardışık gün boyunca intraperitoneal tek doz şeklinde grup C'ye (kontrol grubu) serum fizyolojik, grup P'ye parasetamol ve grup D'ye diklofenak verildi. Sıçanlar belirlenen tarihlerde sakrifiye edildi ve toraks kafesleri bütün olarak çıkarıldı.

Bulgular: Numunelerin makroskopik ve mikroskopik değerlendirmesine göre, parasetamol serum fizyolojiğinkine benzer derecede yapışıklıklar oluşturdu, buna karşın diklofenak iki plevra yaprağı arasındaki istenen yapışıklıkların yoğunluğunu anlamlı şekilde azalttı (p=0.05).

Sonuç: Otolog kan plöredezini takiben anti-enflamatuvar analjeziklerin kullanımı işlemin başarısız sonuçlanmasına yol açabilir.

Anahtar sözcükler: Otolog kan, diklofenak, parasetamol, plöredez, sıçan.

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Correspondence: Serhat Yalçınkaya, MD. Kütahya Sağlık Bilimleri Üniversitesi, Göğüs Cerrahisi Anabilim Dalı, 43100 Kütahya, Turkey.
Tel: +90 274 - 231 66 60 / 2114 e-mail: serhat.yalcinkaya@ksbu.edu.tr

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Pleurodesis following chest tube insertion and drainage is the preferred method of treatment in recurrent fluid and air accumulation between the pleural sheets. This procedure implies strong and widespread adhesion of the visceral and parietal pleura in order to obliterate the potential space in between.[1,2] Pleurodesis may be tried using mechanical, chemical, thermal, and immunological methods. All agents lead to an acute episode of inflammation in the pleural sheets accompanied by a desquamation of mesothelial cells which is reported as the key to a successful pleurodesis in many studies.[3-7] Unfortunately, unsuccessful results are reached with various agents in the same patient, or with the same agent in various patients. Since the procedure is painful, the patients require drugs from one or more of the two major types of analgesics; non-opioids, i.e. paracetamol or non-steroidal anti-inflammatory drugs (NSAIDs), and opioids.[8] A recent study in rats reported that high doses of (2-3 mL/kg) autologous blood administered to the pleural space led to successful pleurodesis.[9] The known advantages of this agent include low cost, being readily available at all times, sterile, and non-allergenic to the patient.[10] To our knowledge, there is no animal study investigating the effects of analgesics on autologous blood pleurodesis (ABP) present in the English literature. Therefore, in this study, we aimed to perform ABP in an animal model and investigate the effects of paracetamol and diclofenac on ABP.

MATERIALS AND METHODS

This study was conducted at Dumlupınar University Medical Faculty Animal Research Laboratory between September 2016 and December 2016. Following the permission from the Dumlupınar University Medical Faculty Ethics Committee for Animal Experiments (permission number: 2016.06.03, permission date: April the 22nd, 2016), we randomly assigned 42 female Wistar Albino rats (aged three months; average weight 275±25 g) to one of the three main groups of 14 rats each: control group (group C), paracetamol group (group P), and diclofenac sodium group (group D). We further divided these groups into two subgroups of seven rats each as seven days group (7) and 21 days group (21) indicating the planned date of sacrifice. All animals received humane care in accordance to the Principles of Laboratory Animal Care, published by the National Society for Medical Research, and to the Guide for the Care and Use of Laboratory Animals, published by the National Academy of Sciences. The rats were kept under well standardized circumstances in air-conditioned rooms with constant temperature, 50±5% humidity, and 12 hours of day/night cycle. Food and water were provided ad libitum under daily surveillance by the authors and veterinary technicians working at the laboratory.

All rats received general anesthesia with ketamine hydrochloride (Alfamine vial, Ege-vet Pharmaceutical Co., Izmir, Turkey) at a dose of 35 mg/kg, and xylazine hydrochloride (Alfazyne vial, Ege-vet Pharmaceutical Co., Izmir, Turkey) at a dose of 5 mg/kg administered intraperitoneally (IP). Following anesthesia, we weighed the rats to determine the amount of venous blood into pre-heparinized insulin syringes at 3 mL/kg dose. The rats were placed in a right decubitus position following blood withdrawal and the left thoracic area was shaved using an electric shaver. Then, we performed a 5 mm incision at the middle portion of the left lateral thoracic wall after aseptic cleaning of the operation site using povidone iodine solution in order to create a space for autologous blood instillation. We administered the withdrawn venous blood through the incision using a 24 G venous catheter placed caudad between the pleural sheets and aspirated the excessive air using the same syringe (Figure 1). Then, we closed the incision using 3/0 polypropylene sutures. We rotated the rats in a circular fashion to make sure that the administered blood was spread all over the pleural

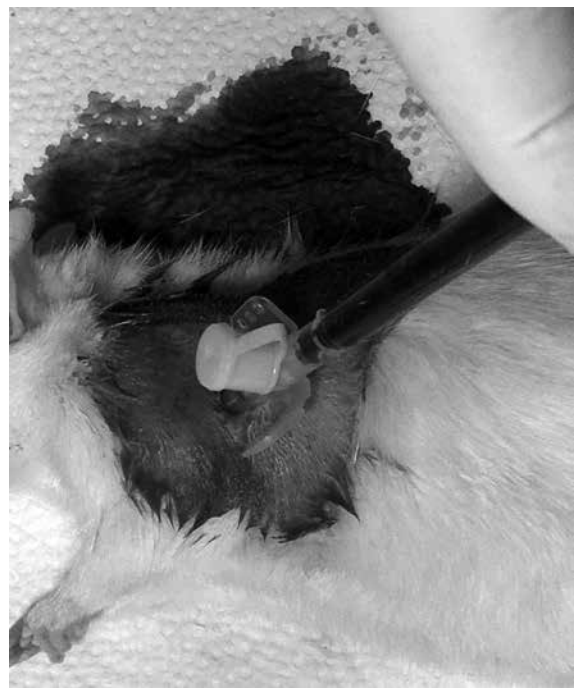

Figure 1. Under general anesthesia, subject was shaved, cleaned and 3 mL/kg autologous blood was administered through thoracotomy incision using a pre-heparinized syringe and 24 G venous catheter.

sheets. The rats were held in separate cages until they fully recovered.

Following the surgery and ABP, we administered a single dose of 2 mL of plain saline solution IP to 14 rats in group C for five days. Then, we sacrificed the rats in group C7 on the seventh and those in group C21 on the 21st postoperative day using cervical dislocation following anesthesia using the same protocol as the operation. One rat from each group was lost before the planned time of sacrifice.

In group P, we administered a single dose of paracetamol in saline solution at 500 mg/kg in 2 mL IP for five days. Then, rats of groups P7 and P21 were sacrificed on proper dates. One rat from each group was lost before the planned time of sacrifice.

In group D, we administered a single dose of diclofenac solution at 10 mg/kg in 2 mL IP for five days. Then, we sacrificed the rats in groups D7 and D21 according to the schedule. Two rats from group D7 and one rat from the latter were lost before the planned time of sacrifice.

We removed the thoracic cage of each rat *en bloc* after dissecting the skin, underlying muscles, and connective tissue (Figure 2). We rinsed the specimens with heparined saline solution and placed these specimens into formaldehyde solution.

Figure 2. *En bloc* excision of thoracic cage after subject is sacrificed.

After five days of soaking in formaldehyde minimum, we renumbered the specimens in order to blind the pathologists to the subgroups and the procedures performed. The pathologist dissected the sternum longitudinally to assess the grade of macroscopical symphysis according to Hurewitz's method.[11] In this classification, grade 0 indicates normal pleural findings without any adhesions; grade 1 indicates a few adhesions in a scattered fashion; grade 2 means that the adhesions are widespread; and grade 3 indicates total symphysis of the pleural sheets with generalized fibrosis.

After choosing the best representing areas of macroscopical adhesions, the incised samples from both the visceral and parietal pleural sheets were fixed in 10% formalin. The samples were then embedded in paraffin and 4 μm-thick slices were placed on slides. For routine microscopical examination, the slides were stained with hematoxylin and eosin. In order to visualize fibrosis in detail, another set of slides were stained with Masson's trichrome stain. The prepared slides were examined using light microscope. The microscopical findings were classified according to degree of histological evidence of fibrosis, and inflammation according to Hurewitz's method.[11] The visceral and parietal pleura mesothelial cells were evaluated as intact, i.e. present, or damaged, i.e. absent. Interpleural adhesion, visceral and parietal pleural involvement, cellularity, and neovascularization were graded. In this classification, grade 0 indicates no sign of fibrosis or inflammation; grade 1 indicates presence of minimal fibrosis, giant cells, plasma cells, and some lymphocytes; grade 2 means moderate fibrosis with increased number of giant cells, plasma cells, eosinophils, neutrophils and lymphocytes; and grade 3 indicates dense fibrosis with more inflammatory cells and microabscess formation.

Statistical analysis

All results were analyzed using MedCalc Statistical Software version 18.9 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2018). The possible statistical relationships between macroscopical and microscopical findings classified as grades revealed within the groups were assessed using chi-square test. A p value less than or equal to 0.05 was accepted as statistically significant.

RESULTS

Due to unplanned losses during the experiment, the total number of rats sacrificed in five of the groups was

Table 1. Macroscopic grades found in subjects

Subject #	Group C7	Group C21	Group P7	Group P21	Group D7	Group D21
1	0	2	1	3	0	0
2	2	2	0	1	0	0
3	1	2	1	3	1	0
4	1	3	1	1	0	1
5	2	2	1	2	0	0
6	1	1	1	2	-*	0
7	-*	-*	-*	-*	-*	-*

* Subjects died unexpectedly.

Table 2. Results of chi-square analysis of macroscopic grades between groups

	Group C7	Group C21	Group P7	Group P21	Group D7	Group D21
Group C7			0.535 (0.301)		0.522 (0.392)	
Group C21				0.655 (0.343)		0.707 (0.050*)
Group P7	0.535 (0.301)				0.243 (0.576)	
Group P21		0.655 (0.343)				0.535 (0.301)
Group D7	0.522 (0.392)		0.243 (0.576)			
Group D21		0.707 (0.050*)		0.535 (0.301)		

* Statistically significant.

Table 3. Microscopic grades found in subjects

Subject #	Group C7	Group C21	Group P7	Group P21	Group D7	Group D21
1	0	2	0	1	0	0
2	1	2	0	2	1	0
3	2	3	1	2	0	1
4	1	1	1	3	0	0
5	1	2	0	1	0	0
6	1	2	1	2	-*	0
7	-*	-*	-*	-*	-*	-*

* Subjects died unexpectedly.

six, whereas it was five in group D21. The results of macroscopic evaluations are listed in Table 1. Although specimens from groups C7 and C21 seemed to present higher macroscopical grades then groups P7, P21, D7 and D21, statistical analysis revealed that the only significant difference was between groups C21 and D21 as listed in Table 2 (p=0.05).

Microscopically, we observed macrophage and lymphocyte infiltration at various levels in visceral and parietal pleura indicating inflammation (Table 3). The degree of fibroblast infiltration was similar in both pleural sheets. Microscopically, loose connective tissue was revealed in lower grades, whereas hypercellularity, dense collagen and fibrin were found in higher grades. Microscopic evaluation revealed the highest grades in groups C7, P7, C21, and P21, and the lowest grades in groups D7 and D21 (Figure 3). The only statistically significant

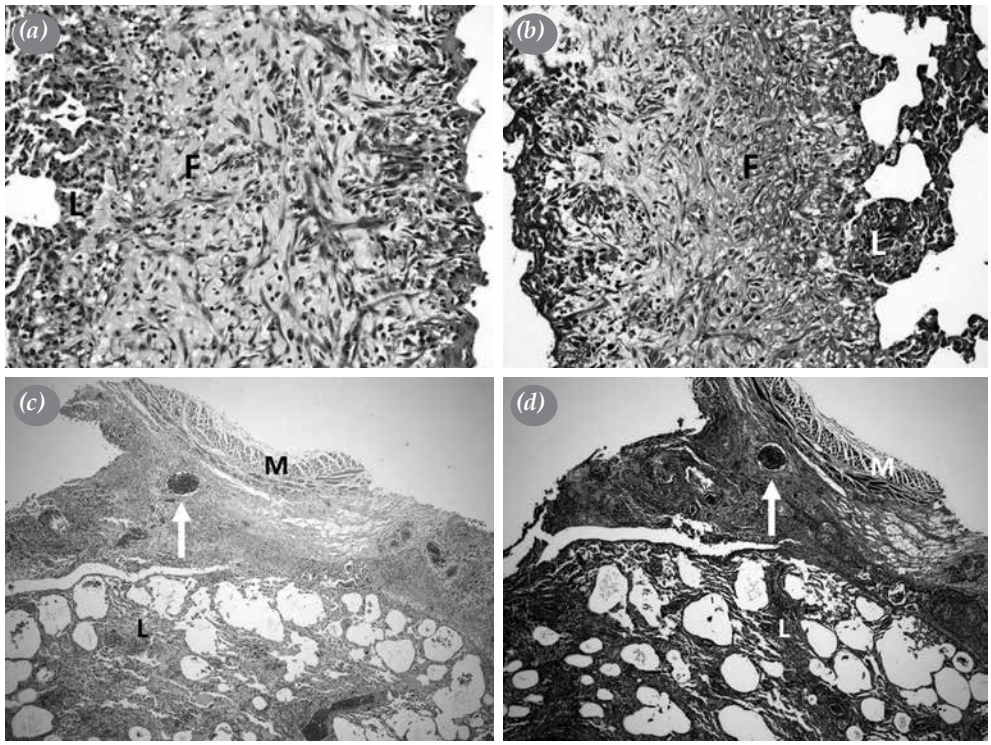

Figure 3. (a) Histopathologic section of subject #C21-1 showing fibrosis (F) consisting of fibroblasts and collagen, hypercellularity, and inflammation of plasmocytes and lymphocytes overlaying lung tissue (L) (grade 2), (H-E×200). **(b)** Masson's trichrome stain showing fibrosis (F) with blue dyed collagen and fibroblasts over lung tissue (L), (Masson's trichrome ×200). **(c)** Histopathologic section of subject #P21-4 showing complete adhesion between pleural sheets including thoracic muscle (M) with fibrosis, dense inflammation, and increased capillary formation (white arrow) surrounding lung (L) (grade 3), (H-E×40). **(d)** Masson's trichrome stain showing blue dyed fibrosis and dense inflammation, muscle fibers (M), capillary formation (white arrow), surrounding lung tissue (L), (Masson's trichrome ×40).

Table 4. Results of chi-square analysis of microscopic grades between groups

	Group C7	Group C21	Group P7	Group P21	Group D7	Group D21
Group C7			0.500 (0.368)		0.378 (0.659)	
Group C21				0.734 (0.136)		0.707 (0.050*)
Group P7	0.500 (0.368)				0.378 (0.361)	
Group P21		0.734 (0.136)				0.438 (0.549)
Group D7	0.378 (0.659)		0.378 (0.361)			
Group D21		0.707 (0.050*)		0.438 (0.549)		

* Statistically significant.

difference was found between groups C21 and D21 as shown in Table 4 (p=0.05).

DISCUSSION

Various agents are used in pleurodesis including silver nitrate, talc, bleomycin, tetracycline, quinacrine, biological glues, and autologous blood.[1,2,9,12,13] As it is readily available, cheap, and it causes no undesired reactions, we preferred autologous blood in this animal study at an effective dose of 3 mL/kg concluded in a former study on rats.[9] Regardless of the agent used, pleurodesis procedure is not always successful. The

success rate basically depends on the surface area of distribution, the irritation caused by the agent, and the intensity of inflammation.[3-7,14] All these features lead to pain sometimes so extreme that the patients receive drugs of various kinds for pain management in the clinical setting. The analgesics may be divided into two major classes: 1) non-opioids including paracetamol, aspirin and various NSAIDs, and 2) opioids.[10] In thoracic surgery, diclofenac is widely used for pain control in various clinical settings. This led us to think about the possible undesirable effects of diclofenac on pleural symphysis, a desired result of many treatments in thoracic surgery. For this reason, we planned to assess the effects of diclofenac on ABP in our study.

Analgesics are reported to affect pleurodesis in many ways. In 1998, Xie et al.[3] concluded that corticosteroids may decrease the effectiveness of talc when used for pleurodesis due to inhibition of inflammatory response in rabbits. In 2004, Lardinois et al.[5] reported that NSAIDs reduce the adhesions following mechanical abrasion during video-assisted thoracic operations. In an experimental pleurodesis model with tetracycline in rabbits, Ors Kaya et al.[6] reported that diclofenac sodium ameliorates the macroscopic pleurodesis score. In another study, NSAIDs reduced adhesions after talc pleurodesis but not after silver nitrate.[7] In a recent study in pediatric patients, Lizardo et al.[15] successfully used NSAID, i.e. ketorolac, in pain management following mechanical pleurodesis using video-assisted thoracic surgery. A thorough search of the English literature available to us revealed no studies comparing the effects of paracetamol and diclofenac on ABP model in rats.

According to our results, diclofenac reduced macroscopic and microscopic pleurodesis grades in ABP, unlike paracetamol. Statistical analysis revealed no significant differences between the results of groups C7 and C21 compared with groups P7 and P21. This may indicate that paracetamol has no undesired effect on ABP and it can be used safely. However, the analysis revealed significant differences between macroscopical and microscopical grades of group C21 compared with group D21 ($p=0.05$). The microscopical finding of desired adhesion, i.e. fibrosis and inflammation, were observed as the lowest in rats in groups D7 and D21. This implies that diclofenac prevents firm symphysis after ABP and it shall not be preferred. The possible mechanism leading to this result may be the strong effect of diclofenac in inhibiting fibroblast proliferation and decreasing the chemotaxis induced by substance P and transforming growth factor-beta.[6] Besides, steroids and similar acting drugs are known to inhibit the early stages of the inflammatory process, which is crucial for a successful pleurodesis, including migration of leukocytes into the area of inflammation, edema formation, and consequent fibrin deposition.[3]

The limitations of our study are the small number of animals, the number of unexpected losses, and the need of artificial pneumothorax in order to install the autologous blood as an agent. Further studies with larger groups and longer periods of follow-up and studies including assessment of pleural fluid for markers and mediators of inflammation may reveal further information about ABP in relation to these analgesics.

In conclusion, according to the macroscopic and microscopic findings of this experimental autologous blood pleurodesis in rats, we conclude that non-steroidal anti-inflammatory drugs may lead to undesired results by reducing the effect of pleurodesis due to anti-inflammatory properties. We believe that preferring this type of drugs in the clinical setting may result in failure of the procedure.

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Declaration of conflicting interests

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REFERENCES

1. Rodriguez-Panadero F, Antony VB. Pleurodesis: state of the art. Eur Respir J 1997;10:1648-54.
2. Dikensoy O, Light RW. Alternative widely available, inexpensive agents for pleurodesis. Curr Opin Pulm Med 2005;11:340-4.
3. Xie C, Teixeira LR, McGovern JP, Light RW. Systemic corticosteroids decrease the effectiveness of talc pleurodesis. Am J Respir Crit Care Med 1998;157:1441-4.
4. Hunt I, Teh E, Southon R, Treasure T. Using non-steroidal anti-inflammatory drugs (NSAIDs) following pleurodesis. Interact Cardiovasc Thorac Surg 2007;6:102-4.
5. Lardinois D, Vogt P, Yang L, Hegyi I, Baslam M, Weder W. Non-steroidal anti-inflammatory drugs decrease the quality of pleurodesis after mechanical pleural abrasion. Eur J Cardiothorac Surg 2004;25:865-71.

6. Ors Kaya S, Bir F, Atalay H, Onem G, Aytekin FO, Saçar M. Effect of diclofenac on experimental pleurodesis induced by tetracycline in rabbits. J Investig Med 2005;53:267-70.
7. Teixeira LR, Vargas FS, Acencio MM, Paz PF, Antonangelo L, Vaz MA, et al. Influence of antiinflammatory drugs (methylprednisolone and diclofenac sodium) on experimental pleurodesis induced by silver nitrate or talc. Chest 2005;128:4041-5.
8. Dureja GP, Iyer RN, Das G, Ahdal J, Narang P. Evidence and consensus recommendations for the pharmacological management of pain in India. J Pain Res 2017;10:709-36.
9. Ozpolat B, Gazyagci S, Gözübüyük A, Ayva S, Atinkaya C. Autologous blood pleurodesis in rats to elucidate the amounts of blood required for reliable and reproducible results. J Surg Res 2010;161:228-32.
10. Karangelis D, Tagarakis GI, Daskalopoulos M, Skoumis G, Desimonas N, Saleptsis V, et al. Intrapleural instillation of autologous blood for persistent air leak in spontaneous pneumothorax- is it as effective as it is safe? J Cardiothorac Surg 2010;5:61.
11. Hurewitz AN, Lidonicci K, Wu CL, Reim D, Zucker S. Histologic changes of doxycycline pleurodesis in rabbits. Effect of concentration and pH. Chest 1994;106:1241-5.
12. Tremblay A, Stather DR, Kelly MM. Effect of repeated administration of low-dose silver nitrate for pleurodesis in a rabbit model. Respirology 2011;16:1070-5.
13. Kahrom H, Aghajanzadeh M, Asgari MR, Kahrom M. Efficacy and Safety of Povidone-iodine Pleurodesis in Malignant Pleural Effusions. Indian J Palliat Care 2017;23:53-6.
14. Teixeira LR, Vargas FS, Acencio MM, Antonangelo L, Novaes NP, Costa JD Jr, et al. Influence of parecoxib (cox-2 inhibitor) in experimental pleurodesis. Respir Med 2009;103:595-600.
15. Lizardo RE, Langness S, Davenport KP, Kling K, Fairbanks T, Bickler SW, et al. Ketorolac does not reduce effectiveness of pleurodesis in pediatric patients with spontaneous pneumothorax. J Pediatr Surg 2015;50:2035-7.