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The effects of preoperative chemotherapy and beta glucan on bronchial anastomosis in rabbits: a preliminary study

Tavşanlarda ameliyat öncesi kemoterapi ve beta glukanın bronş anastomozuna etkileri: Preliminar bir çalışma

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ABSTRACT

Background: This study aims to investigate the effects of an immunostimulant, beta glucan, on the bronchial anastomosis in rabbits that received chemotherapy in preoperative period.

Methods: Forty-five New Zealand male rabbits were used in this study. The rabbits were divided into three groups as group 1 (control), group 2 (chemotherapy), and group 3 (chemotherapy + beta glucan) with 15 rabbits in each group. The left main bronchus was incised completely and then end-to-end anastomosis was performed in all groups. Cisplatin was administered on the preoperative first day and etoposide was administered on the preoperative first, second, and third days in group 2. In group 3, in addition to the same chemotherapy protocol, 10 mg/kg/day beta glucan was administered via enteral route starting from seven days before operation until one day before sacrifications. The rabbits were sacrificed at postoperative third, fifth, and seventh days by groups of five. The bronchial anastomosis lines were removed and tissue sections were stained with hematoxylin and eosin.

Results: In pathological analysis, while there were statistically significant differences between group 1 and group 2 and between group 2 and group 3, there was no difference between group 1 and group 3 in terms of bronchial healing.

Conclusion: The use of beta glucan during chemotherapy treatment may improve bronchial healing at postoperative period.

Keywords: Bronchus; cancer; chemotherapy; sleeve lobectomy.

ÖZ

Amaç: Bu çalışmada ameliyat öncesi dönemde kemoterapi gören tavşanlarda bir immünstimülan olan beta glukanın bronş anastomozuna etkileri araştırıldı.

Çalışma planı: Bu çalışmada kırk beş Yeni Zelanda türü erkek tavşan kullanıldı. Tavşanlar her birinde 15 tavşan olacak şekilde grup 1 (kontrol), grup 2 (kemoterapi) ve grup 3 (kemoterapi + beta glukan) olarak üç gruba bölündü. Tüm gruplarda sol ana bronş tam olarak kesildi ve daha sonra uç uca anastomoz yapıldı. Grup 2'de ameliyat öncesi birinci günde sisplatin ve ameliyat öncesi birinci, ikinci ve üçüncü günde etoposid uygulandı. Grup 3'te aynı kemoterapi protokolüne ilave olarak ameliyattan yedi gün önceden başlanarak sakrifikasyonların bir gün öncesine kadar enteral yolla 10 mg/kg/gün beta glukan verildi. Tavşanlar ameliyat sonrası üçüncü, beşinci ve yedinci günlerde beşerli gruplar halinde sakrifiye edildi. Bronşiyal anastomoz hatları çıkarıldı ve doku kesitleri hematoksilin ve eozin ile boyandı.

Bulgular: Patolojik incelemede grup 1 ve grup 2 arasında ve grup 2 ve grup 3 arasında istatistiksel olarak anlamlı farklılık var iken grup 1 ve grup 3 arasında bronş iyileşmesi açısından farklılık yok idi.

Sonuç: Kemoterapi tedavisi sırasında beta glukan kullanılması ameliyat sonrası dönemde bronşiyal iyileşmeyi düzeltebilir.

Anahtar sözcükler: Bronkus; kanser; kemoterapi; sleeve lobektomi.

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Lung resections have been performed in many diseases, especially in lung cancers for many years. Although these resections can be performed easily in patients who have sufficient pulmonary capacity, parenchyma-saving surgery such as sleeve lobectomy and bronchial sleeve resection come to the agenda in patients with limited lung capacity. Some patients with limited lung capacity are operated after neoadjuvant chemotherapy. However, after the postoperative period, bronchitis may be aggravated due to chemotherapeutic drug since these agents may impair the wound healing in bronchial stump and anastomosis line.[1] This condition may cause bronchopleural or bronchoarterial fistula, prolonged air leak, empyema, and fatal bleeding at postoperative period.[2] Increased bronchial complication rate after neoadjuvant therapy was showed in the literature.[3] Although several techniques supporting the bronchial stump and anastomosis have been described to prevent this complication, a non-surgical technique has not been proposed yet.[4] Therefore, in this experimental study, we aimed to investigate the effects of an immunostimulant, beta glucan, on the bronchial anastomosis in rabbits that received chemotherapy in preoperative period.

MATERIALS AND METHODS

This study was carried out in the Experimental Medicine and Application Center of the Selcuk University between July 2006 and January 2008. Pathology specimens were examined in the Department of Pathology, Meram Medical Faculty, Selcuk University. The study protocol was approved by Selcuk University, Experimental Medicine and Application Center, Laboratory Animals Ethical Board (Approval No: 12.05.2006/22).

In the study, 45 male New Zealand albino rabbits with a mean weight of 302.2±419.75 g were used. These animals were preferred since we would be able to see the bronchial system with more detail and perform the surgical procedure more easily. During the study, ethic principles were respected.

The rabbits were divided into three groups as group 1 (control group), group 2 (chemotherapy group), and group 3 (chemotherapy plus beta glucan group) with 15 rabbits in each group. In addition, each group had three subgroups including five rabbits, which were adjusted according to sacrification periods. All rabbits were fed by standard rabbit diet.

No drug was given to group 1 during pre- and postoperative periods. At the operation day, ketamine 35 mg/kg and xylazine 5 mg/kg were administered intramuscularly, surgical area was shaved and cleaned by povidone-iodine. For prophylaxis, cephazoline sodium 25 mg/kg was given intravenously before surgery. Thereafter, left thoracotomy was performed (Figure 1a). After the left main bronchus was found (Figure 1b), complete incision was performed (Figure 1c). Then, cartilaginous and membranous parts were continuously sutured by 5-0 polypropylene suture (Figure 1d). Finally, thorax was closed and pneumothorax was drained by thoracentesis. After completion of the surgical intervention, same dose cephazoline was given again for prophylaxis and additionally, tramadol 1 mg/kg was administered intravenously for pain control. In the postoperative period, each subgroup was sacrificed at third, fifth, and seventh days, respectively. After the sacrification, anastomosis lines were removed for pathological examination.

Rabbits in group 2 were given cisplatin 4 mg/kg at preoperative first day and etoposide 4.8 mg/kg at preoperative first, second, and third days through ear vein. Dose of the chemotherapeutics was the same with the dose administered to the rats in the experimental study performed by Inoue et al.[5] Surgical procedure, anesthesia, prophylaxis, and analgesia were identical with group 1. Sacrifications were done at postoperative third, fifth, and seventh days and anastomosis lines were removed for pathological examination.

The same chemotherapy protocol was given to group 3. Additionally, beta glucan 10 mg/kg was enterally given through an orogastric tube starting from seven days before operation until one day before sacrifications. Surgical procedure, anesthesia, prophylaxis, and analgesia were the same with the others groups. Sacrifications were done at postoperative third, fifth, and seventh days and anastomosis lines were removed.

Anastomosis lines were fixed by 10% formaldehyde. The sections were stained with hematoxylin and eosin. Thereafter, the slides were examined using Couraud scoring (Table 1).[6] Grade 1 was considered as five, grade 2a as four, grade 2b as three, grade 3a as two, and grade 3b as one to perform statistical analysis (Figure 2a-d).

Statistical analysis

Statistical Package for the Social Sciences version 13.0 for Windows software program (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Descriptive statistics were shown in Tables 1, 2, and 3. Groups were compared using Kruskal-Wallis variance

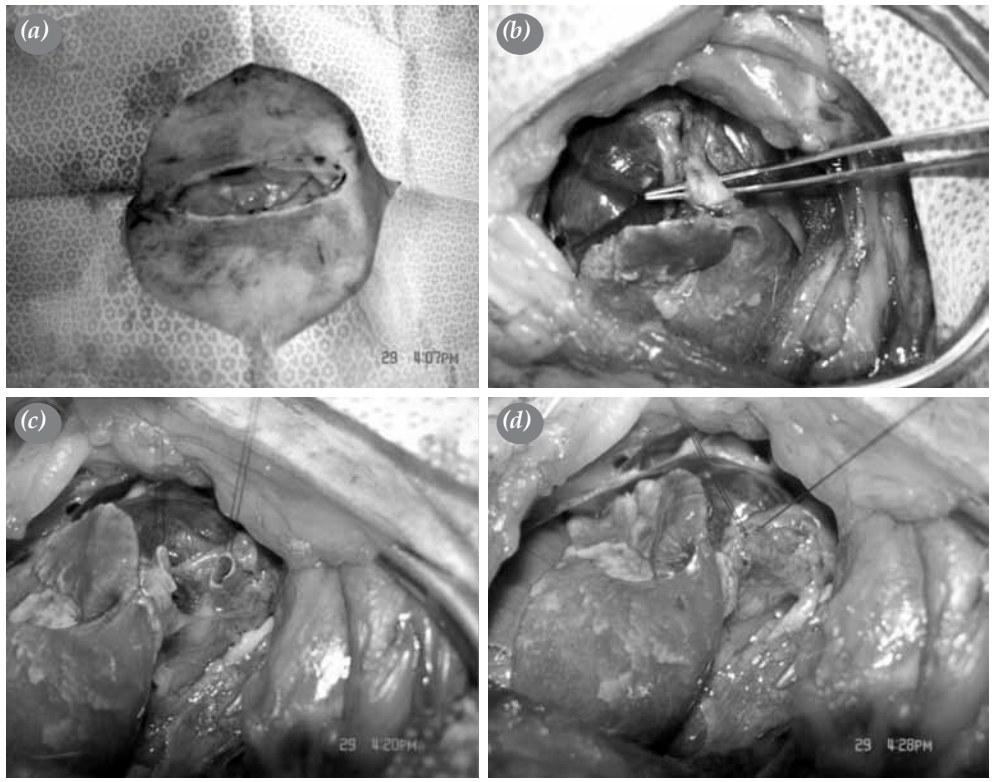

Figure 1. (a) Skin incision for left thoracotomy to rabbits lied on their right side. **(b)** Left main bronchus. **(c)** Complete incision of left main bronchus. **(d)** Appearance after left main bronchus anastomosis.

analysis and a *p* value of <0.05 was considered as significance level. Pairwise comparisons were done using Mann-Whitney U test with Bonferroni correction and a *p* value of <0.05 was considered as significance level.

RESULTS

At postoperative third day, two animals in group 2 developed limited necrosis (Figure 2d), whereas none of the animals in group 1 and group 3 had necrosis. Although we detected better healing in groups 1 and 3, a statistically significant difference was not found (p>0.05). Complete and near-complete epithelization and healing were seen in group 1 and group 3 (Figure 2a, b).

Table 1. Grading system for bronchial healing[6]

Grade 1	Complete mucosal healing
Grade 2a	Complete primary healing without necrosis partial primary mucosal healing
Grade 2b	Complete primary healing without necrosis No primary mucosal healing
Grade 3a	Limited focal necrosis (<5 mm)
Grade 3b	Extensive necrosis

At postoperative fifth day, one animal in group 2 developed limited necrosis, whereas necrosis was not observed in other groups. Incomplete wound healing without epithelization was detected in all groups (Figure 2c). An evaluation at postoperative seventh day revealed that three animals in group 1 and one animal in group 3 healed completely (Figure 2a). In group 2, complete recovery was not observed in any of the postoperative periods.

Minimum, maximum, and median values of the pathological scores of groups obtained at postoperative third, fifth, and seventh days are shown in Table 2. When all groups were collectively evaluated, wound healing was significant at postoperative seventh day (p<0.05). When the groups were dichotomously evaluated, no statistically significant difference was detected between the groups at postoperative third and fifth days (p>0.05). An evaluation at postoperative seventh day showed a significant difference between group 1 and group 2 in terms of bronchial healing. While there was no statistically significant difference between group 1 and group 3 (p>0.05), there was a statistically significant difference between group 2 and group 3 (p<0.05), (Table 3).

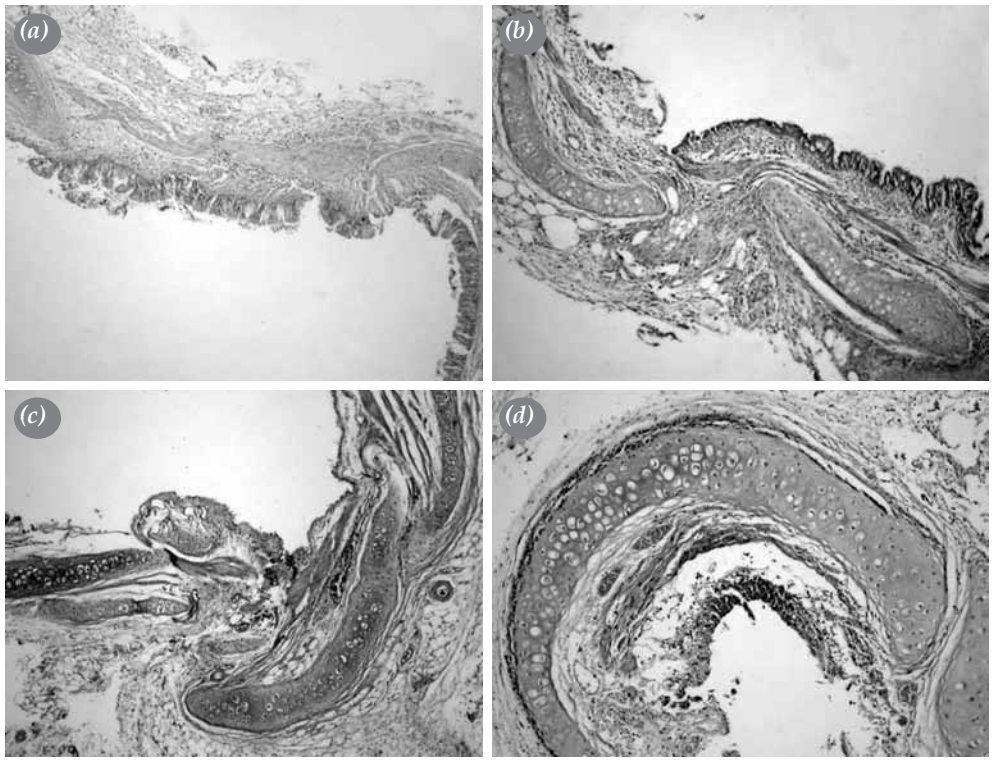

Figure 2. (a) Grade 1, complete epithelization and healing (H-E x 40). **(b)** Grade 2a, near-complete wound healing with epithelization in patches and less inflammatory cell (H-E x 40). **(c)** Grade 2b, incomplete wound healing without epithelization (H-E x 40). **(d)** Grade 3a, necrosis on endobronchial surface and mild healing findings (H-E x 40).

DISCUSSION

Thanks to recent technological developments and increasing knowledge, thoracic surgery has been performed more frequently. Meanwhile, lung cancer is increasingly diagnosed due to increased incidence of smoking and environmental exposure. Surgery is still the most widely accepted method for the treatment of operable lung cancer. Chemotherapy, radiotherapy, and supportive therapy are commonly used in the treatment of lung cancer at advanced stages. However, the toxicity of chemotherapy on the tissues remains to be an important problem and data about the effect of the chemotherapy on post-surgical bronchial healing have not been clearly obtained yet.

Although the effect of acute radiotherapy on bronchial anastomosis has been experimentally and clinically demonstrated, there are limited numbers of studies about the effect of the preoperative chemotherapy on postsurgical tracheobronchial wound healing.[7,8] Inoue et al.[5] demonstrated that preoperative chemotherapy impaired bronchial healing in their experimental study.

This condition may be linked with subgroups of leukocytes, which are peripheral blood cells, including neutrophils, monocytes, lymphocytes, eosinophils, and basophils. Macrophages originate from the monocytes. Etoposide and cisplatin may impair the bronchial healing by causing leukopenia and macrophage deficiency in the healing tissue.[9] In our study, we found that group 2 had a significantly impaired bronchial wound healing.

Beta glucan is obtained from the cell wall of Saccharomyces cerevisiae, commonly known as Baker's yeast. This agent ensures macrophage activation and accelerates wound healing.[10-12] Nevertheless, to our knowledge, there is no study in the literature that investigated the effect of beta glucan on bronchial healing. We investigated the effect of beta glucan on bronchial healing since neoadjuvant chemoradiotherapy, which may increase bronchial complication rates, is considered in patients with lung cancer.

In another experimental study, Breivik et al.[13] demonstrated that soluble beta 1.3/1.6 glucan may prevent periodontal disease. In their study, the

Table 2. Pathological scores of the groups

	Group 1			Group 2			Group 3		
Postoperative days	3	5	7	3	5	7	3	5	7
Median	3.00	3.00	4.00	3.00	3.00	3.00	3.00	4.00	4.00
Minimum	3.00	3.00	4.00	2.00	2.00	3.00	3.00	3.00	4.00
Maximum	3.00	3.00	5.00	3.00	4.00	3.00	3.00	4.00	5.00

Table 3. Pairwise comparison of the pathological scoring values between the groups

	Group 1-Group 2			Group 1-Group 3			Group 2-Group 3		
Postoperative days	3	5	7	3	5	7	3	5	7
P value (2-tailed)	0.134	1.00	0.004	1.00	0.050	1.00	0.134	0.166	0.004

ligation of the neck of the second right maxillar molar tooth by sterile silk resulted in the retention of oral microorganisms. This protective effect of beta glucan was attributed to the activation of the immune system and they reported that the group treated with beta glucan had higher level of interleukin 10 compared to control group. Furthermore, beta glucan can increase the macrophage count in the early stage of wound healing and accelerate wound healing in diabetic mice.[11] Oral and systemic intake of beta glucan has a protective effect against lipopolysaccharide-related shock and organ damage in rats.[14]

Similarly with previous experimental animal models, we also performed the sacrifications at postoperative third, fifth, and seventh days and consistently to the reported time interval. Histopathological evaluation was performed using the Couraud scoring system, considering the mucosal healing grade and presence of necrosis (Table 2). At postoperative third day, two animals in group 2 developed limited necrosis, whereas none of the animals in group 1 and in group 3 had necrosis. Although bronchial healing was better in these groups, no statistically significant difference was detected ($p>0.05$).

At postoperative day 5, one animal in the group 2 showed limited necrosis, whereas necrosis was not observed in other groups. In the evaluation done at postoperative day 7, three animals in group 1 and one animal in the group 3 showed complete healing. In group 2, complete recovery was observed in none of the postoperative periods. When all groups were collectively evaluated, wound healing was significant at postoperative day 7 ($p<0.05$).

When the groups were dichotomously evaluated, there was not statistically significant difference between the groups at postoperative days 3 and 5 ($p>0.05$). In the evaluation done at postoperative day 7, there was significant difference between group 1 and group 2 in bronchial healing. While there was no statistically significant difference between group 1 and group 3 ($p>0.05$), there was a statistically significant difference between the group 2 and group 3 ($p<0.05$). The facts that necrosis was observed only in group 2 and complete healing was shown in group 1 and group 3 were the strongest findings indicating that chemotherapy histopathologically impairs bronchial healing and beta glucan has a favorable effect on wound recovery.

Similarly, Shirafuji et al.,[9] obtained the same results for chemotherapy. According to the researchers, chemotherapy impaired bronchial healing and this risk may be reduced by increasing the interval between chemotherapy and surgical intervention. Wound healing is impaired in patients with cancer due to the disposition of the disease and chemotherapeutics used, especially by suppressing the inflammatory response.[15,16]

Many favorable results have been reported for beta glucan. It has been used in combination with chemotherapy for the treatment of cancer for its immunostimulant effect. Beta glucan is efficient in the treatment of lymph node metastasis of ovarian cancer and advanced breast cancer as it activates peripheral blood monocytes and stimulates the proliferation.[17,18] Furthermore, beta glucan can improve the survival in non-resectable or recurrent gastric cancers.[19,20]

In conclusion, based on the results we obtained in experimental complete bronchial section similar to sleeve bronchial resection, we concluded that preoperative chemotherapy impairs bronchial healing. On the other hand, concomitant use of chemotherapy and beta-glucan led to statistically significantly improved healing parameters. However, due to the lack of previous studies that investigated the effect of beta glucan on bronchial healing, we believe that our results should be supported by further comprehensive studies with larger sample size.

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