J Surg Med. 2020;4(8):689-692.
DOI: 10.28982/josam.780681

Research article
Araştırma makalesi

Journal of Surgery and Medicine
e-ISSN=2602-2079

Investigation of the effects of tadalafil and telmisartan in bleomycin-induced pulmonary fibrosis on rats

Tadalafil ve telmisartanın bleomisine bağlı akciğer fibrozisi üzerindeki etkilerinin sıçanlarda incelenmesi

İlke Onur Kazaz [1], Güner Kemal Özgür [1], Ümit Çobanoğlu [2], Nuri İhsan Kalyoncu [3], Ersagun Karagüzel [1], Murat Topbaş [4], Hüseyin Eren [5], Seher Nazlı Kazaz [6], Rasin Özyavuz [1]

[1] Department of Urology, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Turkey
[2] Department of Pathology, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Turkey
[3] Department of Pharmacology, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Turkey
[4] Department of Public Health, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Turkey
[5] Department of Urology, Research and Training Hospital, Recep Tayyip Erdoğan University, 53020, Rize, Turkey
[6] Department of Medical Oncology, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Turkey

ORCID ID of the author(s)
İOK: 0000-0002-2106-0016
GKÖ: 0000-0002-6872-2355
ÜÇ: 0000-0001-6429-427X
NİK: 0000-0002-7362-6560
EK: 0000-0003-4270-0147
MT: 0000-0002-0660-197X
HE: 0000-0002-1406-8781
SNK: 0000-0002-8181-000X
RÖ: 0000-0002-7362-6560

Corresponding author/Sorumlu yazar:
İlke Onur Kazaz
Address/Adres: Karadeniz Teknik Üniversitesi Tıp Fakültesi, Üroloji Anabilim Dalı, Ortahisar, Trabzon, Türkiye
E-mail: drilke@gmail.com.tr

Ethics Committee Approval: This study was approved by Karadeniz Technical University Animal Care and Ethical Committee (Date: 5/25/2011, Protocol number: 2011/22). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.
Etik Kurul Onayı: Bu çalışma, Karadeniz Teknik Üniversitesi Hayvan Deneyleri Etik Kurulu (Tarih: 25.05.2011, Protokol numarası: 2011/22) tarafından onaylanmıştır. İnsan katılımcıların katıldığı çalışmalardaki tüm prosedürler, 1964 Helsinki Deklarasyonu ve daha sonra yapılan değişiklikler uyarınca gerçekleştirilmiştir.

Conflict of Interest: No conflict of interest was declared by the authors.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.

Financial Disclosure: The authors declared that this study has received no financial support.
Finansal Destek: Yazarlar bu çalışma için finansal destek almadıklarını beyan etmişlerdir.

Published: 8/30/2020
Yayın Tarihi: 30.08.2020

Abstract

Aim: Pulmonary toxicity related to bleomycin, an antitumor drug used in the treatment of several malignancies, is a challenge, and studies to find out molecules to prevent it are ongoing. In this study, we aimed to investigate the effectiveness of telmisartan and tadalafil in an experimental rat model of bleomycin-induced lung fibrosis.

Methods: A total of 32 male rats were divided into four groups: Control group, Bleomycin group, Bleomycin-plus-Tadalafil group and Bleomycin-plus-Telmisartan group. Lung fibrosis was achieved by intratracheal administration of bleomycin, and the same procedure was performed to the control group, but saline was substituted for bleomycin. Tadalafil and telmisartan were administered with an orogastric catheter for 14 days. Tissue malondialdehyde levels (MDA) were determined using colorimetric methods. Masson's trichrome staining was used in the histological examination of the tissue samples.

Results: The fibrosis scores of bleomycin-plus-tadalafil and bleomycin-plus-telmisartan groups were lower than that of the bleomycin group (P=0.007 and P=0.007, respectively). MDA levels did not differ among study groups.

Conclusion: Tadalafil and telmisartan were found to decrease fibrosis scores, which were increased with bleomycin, concluding that pulmonary toxicity was related to multiple processes and preventable.

Keywords: Pulmonary fibrosis, Bleomycin, Telmisartan, Tadalafil

Öz

Amaç: Farklı malignitelerin tedavisinde kullanılan bir antitümör ilaç olan bleomisin ile ilişkili pulmoner toksisite, önemli bir sorundur ve bu toksisiteyi önleyecek molekülleri bulmak için çalışmalar devam etmektedir. Bu deneysel çalışmada, ratlarda, bleomisinle indüklenen akciğer fibrozisinde telmisartan ve tadalafilin etkinliğini araştırmayı amaçladık.

Yöntemler: 32 erkek rat dört gruba ayrıldı: Kontrol grubu, Bleomisin grubu, Bleomisin+Tadalafil grubu, Bleomisin+Telmisartan grubu. Akciğer fibrozisi intratrakeal bleomisin uygulaması ile oluşturulurken aynı prosedür kontrol grubunda bleomisin yerine salin kullanılarak uygulandı. Tadalafil ve telmisartan gruplara göre 14 gün boyunca orogastrik kateter vasıtasıyla verildi. Doku MDA düzeyleri kalorimetrik yöntemler kullanılarak ölçüldü. Masson trikrom boyama uygulanarak histolojik değerlendirme yapıldı.

Bulgular: Bleomisin+tadalafil ve bleomisin+telmisartan gruplarının fibrozis skorları bleomisin grubuna göre daha düşüktü (P=0,007 ve P=0,007, sırasıyla). Gruplar arasında MDA düzeyleri açısından farklılık görülmedi.

Sonuç: Tadalafil ve telmisartanın bleomisine bağlı artan fibrozis skorlarını düşürdüğü ve pulmoner toksisitenin birden fazla süreçle ilişkili olduğu ve önlenebilir olduğu kanısındayız.

Anahtar kelimeler: Pulmoner fibrozis, Bleomisin, Telmisartan, Tadalafil

How to cite/Atıf için: Kazaz İO, Özgür GK, Çobanoğlu Ü, Kalyoncu Nİ, Karagüzel E, Topbaş M, Eren H, Kazaz SN, Özyavuz R. Investigation of the effects of tadalafil and telmisartan in bleomycin-induced pulmonary fibrosis on rats. J Surg Med. 2020;4(8):689-692.

Introduction

Bleomycin is an antitumor antibiotic that is used to treat a wide variety of malignancies, predominantly, germ cell tumors and Hodgkin lymphomas. However, pulmonary toxicities, interstitial pulmonary fibrosis being the most life-threatening variant, can be observed in around ten percent of patients receiving the drug [1,2]. The phosphodiesterase type 5 (PDE 5) inhibitors cause vasodilation in many tissues by blocking the degradation of cyclic guanosine monophosphate (cGMP), which results in prolongation of the action of various mediators, such as nitric oxide (NO). This effect of PDE 5 inhibitors are mostly seen in the penis and lung. There are diverse studies which demonstrated antiapoptotic, antifibrotic and anti-inflammatory effects of PDE 5 inhibitors in different tissues [3-6]. Angiotensin-II receptor blockers (ARBs) are used to treat high blood pressure, heart failure and diabetic kidney disease. Anti-apoptotic and antifibrotic effects of ARBs in lungs, heart and kidneys have been demonstrated in several studies [7-10]. Molecules which harbor antifibrotic potential are currently being studied in experimental settings, and we aimed to investigate the effects of tadalafil, a phosphodiesterase type-5 inhibitor, and telmisartan, an angiotensin receptor blocker, in bleomycin-treated rat model.

Materials and methods

The study was carried out with the approval of Karadeniz Technical University Animal Care and Ethical Committee (Date: 5/25/2011, Protocol number: 2011/22). A total of 32 male Sprague-Dawley rats, weighing between 250-300 grams, were used. The rats were randomized into four groups as follows: Control group, Bleomycin group, Bleomycin-plus-Tadalafil group and Bleomycin-plus-Telmisartan group. All rats were kept in steel cages at a room temperature of 22°C and fed on standard chow pellet diet, with *ad libitum* access to tap water.

After 8 hours of fasting, the rats were anesthetized with intraperitoneal injection of 50 mg/kg ketamine and 10 mg/kg xylazine. The Pulmonary Fibrosis Model was achieved by intratracheal administration of 0.2 ml of the designated solution (bleomycin 5 mg/kg in saline in all groups excluding controls, and 0.2 ml saline only in the control group) on day 0. In the following 14 days, saline, tadalafil or telmisartan were administered to the related groups through an orogastric catheter (10 mg/kg of tadalafil or 10 mg/kg telmisartan). On day 15, all rats were sacrificed, and their lungs were excised. The weights of right and left lungs of all rats were noted and the right lungs were used for histopathological examination, whereas the left lungs were used for biochemical assays.

Histopathological examination

The right lungs were fixed in 10% formaldehyde, after which paraffin blocks were prepared for light microscopic investigations. 3 μm-thick serial sections were made with a microtome and stained with hematoxylin-eosin and Masson's trichrome for evaluation of general morphology and fibrosis. Then the sections were analyzed under a light microscope (Olympus BX 51, Tokyo, Japan) at a high magnification by a histologist blinded to the animal groups. The histological sections were graded for pulmonary fibrosis, scores ranging from 0 (normal lung) to 8 (total fibrosis), using the grading system described by Aschcroft et al. (Grade 0 = Normal lung, Grade 1 = Minimal fibrous thickening of alveolar or bronchiolar walls, Grade 2-3 = Moderate thickening of walls obvious damage to lung architecture, Grade 4-5 = Increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses, Grade 6-7 = Severe distortion of structure and large fibrous areas, "honeycomb lung" is placed in this category and Grade 8 = Total fibrous obliteration of the field) [11] .

Biochemical assay (tissue MDA levels)

Tissue levels of MDA were measured spectrophotometrically with the method described by Uchiyama and Mihara on the thiobarbituric acid reactive substance (TBARS) [12]. Left lung tissues of the rats were cut on ice molds and weighed at 100 mg on a precision scale (Mettler Toledo AB 204-S Greifensee, Switzerland). 1 mL of cold homogenization buffer (1.15% KCl and 0.05% Triton X-100 solution) was placed and homogenized at 5000 rpm in a homogenizer (IKA- ultra turrax T 18, Staufen, Germany) for 30 s in a cold environment. After homogenization, samples were centrifuged at 1800g for 10 min. The supernatant fractions were analyzed at 512 nm by microplate reader (Molecular devices Versa Max, California, United States). Tetramethoxypropane was used as a standard, and MDA levels were calculated as nanomoles per gram wet tissue.

Statistical analysis

Data were expressed as median (interquartile range for 25-75 %). SPSS (version 23.0, Chicago, IL, USA) was used for statistical analysis of the data, where the groups were compared by Kruskal-Wallis analysis of variance (post-hoc evaluations were done by Mann-Whitney U test). A *P*-value of less than 0.05 was considered significant.

Results

The weights of the left lungs were similar in all groups. The weights of right lungs in two groups (bleomycin group and bleomycin-plus-tadalafil group) were higher compared to controls ($P=0.015$ and $P=0.003$, respectively).

The histopathological analysis of lung tissues from untreated, tadalafil and telmisartan-treated bleomycin groups revealed that both drugs had moderately protective effects on lung injury (Figure 1). The fibrosis scores of the bleomycin group were higher compared to the control group ($P<0.05$). The fibrosis scores of bleomycin-plus-tadalafil and bleomycin-plus-telmisartan groups were lower than the bleomycin group ($P=0.007$ and $P=0.007$, respectively). The scores from these two groups were comparable to the scores of the control group.

Analysis for tissue MDA levels did not show any difference for the studied groups. The results of the statistical analysis are presented in Table 1.

Table 1: A comparison of lung weight, MDA levels and fibrosis score in the groups

Variables	Control (n:8)	Bleomycin (n:8)	Bleomycin/ tadalafil (n:8)	Bleomycin/ telmisartan (n:8)	*P*-value
Lung Weight					
Right lung weight (mg)	1100 (1000-1275)	1450 (1300-1575)[a]	1550 (1325-1950)[a]	1300 (1125-1475)	0.015
Left lung weight (mg)	800 (625-1000)	850 (800-1050)	950 (800-1000)	1000 (850-1075)	0.421
Biochemical Variable					
MDA (nmol/mg wet tissue)	4.80 (4.32-5.44)	5.22 (4.31-5.70)	4.35 (3.43-4.95)	4.56 (4.19-5.60)	0.713
Histopathologic Variable					
Fibrosis Score	3.0 (1.0-3.0)	3.0 (1.0-6.50)[a]	1.0 (1.0-3.0)[b]	1.0 (1.0-3.0)[b]	0.018

Data were expressed as median (interquartile range for 25-75%). *P*-values according to Kruskal-Wallis Test, post hoc Mann Whitney U test. [a] $P<0.05$ compared with control group; [b] $P<0.05$ compared with Bleomycin group

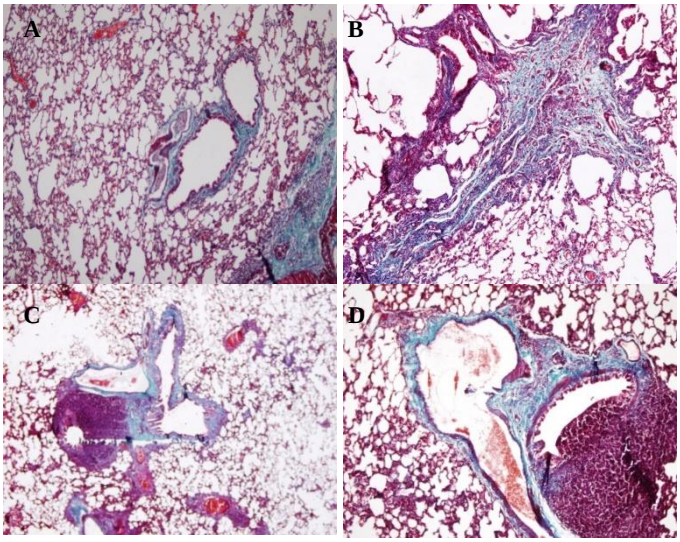

Figure 1: A: Normal appearance of lung parenchyma in control group B: Bleomycin group C: Bleomycin /Tadalafil Group D: Bleomycin/Telmisartan Group (Masson's trichrome staining, original magnifications ×40)

Discussion

Bleomycin is a prevalent drug in regimens for the treatment of testicular germ cell tumors (Bleomycin-Etoposide-Cisplatin (BEP), Cisplatin-Vinblastine-Bleomycin or Carboplatin-Vinblastine-Bleomycin) [13,14]. Pulmonary complications were found in 5% to 16% of patients treated with bleomycin for germ cell cancers [15]. In a study, it has been shown that long-term pulmonary complications persisted in 8% of the patients who received three cycles of BEP for germ cell tumors [16]. The pathophysiological process leading to pulmonary toxicity has not been clarified but oxidative damage, deficiency of bleomycin hydrolase, genetic susceptibility and inflammatory cytokines are thought to be relevant. Bleomycin hydrolase is known to inactivate bleomycin and decrease its effects [2]. Oxidative damage or chronic inflammation and fibrosis of pulmonary interstitial tissues may also play a role in bleomycin-related pulmonary injury. Bleomycin-induced oxidative stress, DNA breakage and epithelial cell obliteration provoke the accumulation of activated inflammatory cells, which release proinflammatory cytokines and growth factors. These fibrotic cytokines such as TNF-α, IL-1 and TGF-β are thought to increase inflammatory response, leading to myofibroblast activation, collagen deposition and remodeling progress. Imbalance between oxidants and antioxidants may play a significant role in pulmonary inflammation that induce fibrosis [17,18].

Antifibrotic and/or anti-inflammatory effects of PDE-5 inhibitors were shown in experimental studies. In a study, Marcus et al. showed that vardenafil inhibited myofibroblast transformation, collagen gel contraction and extracellular matrix production in a rat model of Peyronie's Disease [19]. In other studies, it was shown that tadalafil can reduce hepatic and circulating levels of TNF-α and limit the upregulation of pro-inflammatory cytokines [20-22]. Yildirim et al. reported that sildenafil lowered MDA levels and corrected antioxidant glutathione in a pulmonary fibrosis model (bleomycin-treated rats) [23]. However, our results were different from that of Yildirim's study, showing no significant changes in MDA levels. On the other hand, histopathologically, our results concluded that tadalafil was beneficial in preventing fibrosis in lungs.

TGF-β is an important profibrotic growth factor, controlling cell growth, extracellular matrix, and collagens. The role of reactive oxygen species and angiotensin on this process is well studied. Thus, angiotensin receptor blocked by telmisartan, is thought to be a potential target for preventing fibrosis in lungs [24]. Our histopathological findings were parallel to the hypothesis. We found that telmisartan can ameliorate the fibrosis in lungs. In a study where TGF- β levels were investigated, Waseda and colleagues showed that olmesartan, another angiotensin receptor blocker, had antifibrotic effects in rats with pulmonary fibrosis due to bleomycin, and they reported a decrease in TGF- β levels [8].

Limitations

The most important limitation of our study is the inadequate biochemical parameters. Another limitation of this research is that in vivo studies cannot be predicted in terms of possible effects.

Conclusion

We conclude that bleomycin-induced pulmonary fibrosis may be related to multiple processes and preventable. Our results with a phosphodiesterase type-5 inhibitor tadalafil and an angiotensin receptor blocker telmisartan may be considered promising and direct us to molecular studies to find the pathways related to pulmonary complications.

References

1. O'Sullivan JM, Huddart RA, Norman AR, Nicholls J, Dearnaley DP, Horwich A. Predicting the risk of bleomycin lung toxicity in patients with germ-cell tumours. Ann Oncol. 2003;14:91–6.
2. Sleijfer S. Bleomycin-Induced Pneumonitis. Chest. 2001;120:617–24.
3. Bae EH, Kim IJ, Joo SY, Kim EY, Kim CS, Choi JS, et al. Renoprotective effects of sildenafil in DOCA-salt hypertensive rats. Kidney Blood Press Res. 2012;36:248–57.
4. Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, et al. Long-Term Continuous Treatment with Sildenafil Ameliorates Aging-Related Erectile Dysfunction and the Underlying Corporal Fibrosis in the Rat1. Biol Reprod. 2007;76:915–23.
5. Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, et al. Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction that occurs after cavernosal nerve resection. BJU Int. 2008;101:203–10.
6. Iacono F, Prezioso D, Somma P, Chierchia S, Galasso R, Micheli P. Histopathologically proven prevention of post-prostatectomy cavernosal fibrosis with sildenafil. Urol Int. 2008;80:249–52.
7. Hao J, Wang B, Jones SC, Jassal DS, Dixon IMC. Interaction between angiotensin II and Smad proteins in fibroblasts in failing heart and in vitro. Am J Physiol-Heart Circ Physiol. 2000;279:H3020–30.
8. Waseda Y, Yasui M, Nishizawa Y, Inuzuka K, Takato H, Ichikawa Y, et al. Angiotensin II type 2 receptor antagonist reduces bleomycin-induced pulmonary fibrosis in mice. Respir Res. 2008;9:43.
9. Wamsley-Davis A, Padda R, Truong LD, Tsao CC, Zhang P, Sheikh-Hamad D. AT 1A -mediated activation of kidney JNK1 and SMAD2 in obstructive uropathy: preservation of kidney tissue mass using candesartan. Am J Physiol-Ren Physiol. 2004;287:F474–80.
10. Liu S-S, Wang H-Y, Tang J-M, Zhou X-M. Hypoxia-Induced Collagen Synthesis of Human Lung Fibroblasts by Activating the Angiotensin System. Int J Mol Sci. 2013;14:24029–45.
11. Ashcroft T, Simpson JM, Timbrell V. Simple method of estimating severity of pulmonary fibrosis on a numerical scale. J Clin Pathol. 1988;41:467–70.
12. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978;86:271–8.
13. Motzer RJ, Nichols CJ, Margolin KA, Bacik J, Richardson PG, Vogelzang NJ, et al. Phase III Randomized Trial of Conventional-Dose Chemotherapy With or Without High-Dose Chemotherapy and Autologous Hematopoietic Stem-Cell Rescue As First-Line Treatment for Patients With Poor-Prognosis Metastatic Germ Cell Tumors. J Clin Oncol. 2007;25:247–56.
14. Huddart RA, Gabe R, Cafferty FH, Pollock P, White JD, Shamash J, et al. A Randomised Phase 2 Trial of Intensive Induction Chemotherapy (CBOP/BEP) and Standard BEP in Poor-prognosis Germ Cell Tumours (MRC TE23, CRUK 05/014, ISRCTN 53643604). Eur Urol. 2015;67:534–43.
15. Necchi A, Miceli R, Oualla K, Sonpavde G, Giannatempo P, Raggi D, et al. Effect of Bleomycin Administration on the Development of Pulmonary Toxicity in Patients With Metastatic Germ Cell

Tumors Receiving First-Line Chemotherapy: A Meta-Analysis of Randomized Studies. Clin Genitourin Cancer. 2017;15:213-220.e5.
16. de Wit R, Roberts JT, Wilkinson PM, de Mulder PHM, Mead GM, Fosså SD, et al. Equivalence of Three or Four Cycles of Bleomycin, Etoposide, and Cisplatin Chemotherapy and of a 3- or 5-Day Schedule in Good-Prognosis Germ Cell Cancer: A Randomized Study of the European Organization for Research and Treatment of Cancer Genitourinary Tract Cancer Cooperative Group and the Medical Research Council. J Clin Oncol. 2001;19:1629–40.
17. Mansour HM, Salama AAA, Abdel-Salam RM, Ahmed NA, Yassen NN, Zaki HF. The anti-inflammatory and anti-fibrotic effects of tadalafil in thioacetamide-induced liver fibrosis in rats. Can J Physiol Pharmacol. 2018;96:1308–17.
18. Sriram N, Kalayarasan S, Sudhandiran G. Epigallocatechin-3-gallate augments antioxidant activities and inhibits inflammation during bleomycin-induced experimental pulmonary fibrosis through Nrf2–Keap1 signaling. Pulm Pharmacol Ther. 2009;22:221–36.
19. Ilg MM, Mateus M, Stebbeds WJ, Milenkovic U, Christopher N, Muneer A, et al. Antifibrotic Synergy Between Phosphodiesterase Type 5 Inhibitors and Selective Oestrogen Receptor Modulators in Peyronie's Disease Models. Eur Urol. 2019;75:329–40.
20. Rocha F, Silva Jr F, Leite A, Leite A, Girão V, Castro R, et al. Tadalafil analgesia in experimental arthritis involves suppression of intra-articular TNF release. Br J Pharmacol. 2011;164:828–35.
21. Varma A, Das A, Hoke NN, Durrant DE, Salloum FN, Kukreja RC. Anti-Inflammatory and Cardioprotective Effects of Tadalafil in Diabetic Mice. PLoS ONE. 2012;7:e45243.
22. Dina AAL, Walaa YA, Olfat GS, Lobna OE. Evaluation of the colo-protective effects of tadalafil in an experimental model of ulcerative colitis in rats. Afr J Pharm Pharmacol. 2017;11:385–93.
23. Yildirim A, Ersoy Y, Ercan F, Atukeren P, Gumustas K, Uslu U, et al. Phosphodiesterase-5 inhibition by sildenafil citrate in a rat model of bleomycin-induced lung fibrosis. Pulm Pharmacol Ther. 2010;23:215–21.
24. Shang P, Liu T, Liu W, Li Y, Dou F, Zhang Y, et al. Telmisartan improves vascular remodeling through ameliorating prooxidant and profibrotic mechanisms in hypertension via the involvement of transforming growth factor-β1. Mol Med Rep. 2017;16:4537–44.

This paper has been checked for language accuracy by JOSAM editors.
The National Library of Medicine (NLM) citation style guide has been used in this paper.