The inflammatory effect of captopril on rabbit trachea: a histopathological examination

Kaptoprilin tavşan trakeası üzerine olan inflamatuvar etkisi: Histopatolojik inceleme

Ebru Ünsal, M.D.,[1] Funda Demirağ, M.D.,[2] Abuzer Acar, M.D.,[3] Müjgan Güler, M.D.,[1] Atalay Çağlar, M.D.,[4] Nermin Çapan, M.D.[1]

Departments of [1]Chest Diseases and [2]Pathology, Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara; [3]Veterinary Faculty of Kocatepe University, Afyon; [4]Department of Statistics, Kafkas University, Kars

Objectives: Several mechanisms have been proposed to explain angiotensin converting enzyme (ACE) inhibitor-induced cough, but the exact mechanism is not known. In this study, we aimed to examine the histopathological changes in rabbit trachea after captopril administration.

Study design: Twenty-eight male Angora rabbits were divided into four groups equal in number. Group 1 was the control group. Group 2, 3, and 4 received oral captopril (1 mg/kg) once daily for seven days through an oropharyngeal cannula. Under intramuscular ketamine and xylazine hydrochloride anesthesia, the tracheas were dissected on the first, seventh, and 21st days of captopril withdrawal in group 2, 3, and 4, respectively. The tracheas of the control group were dissected on the same day as group 4. Tracheal sections were stained with hematoxylin-eosin and examined under the light microscope. Inflammation was evaluated by a quantitative scoring system.

Results: On the first day (group 2) and seventh day (group 3) of captopril withdrawal, there was significant inflammation in the tracheas compared with the control group (p=0.002, p=0.001, respectively), without any significant difference between group 2 and 3 (p=0.872). On the 21st day (group 4), inflammation reduced significantly and there was no significant difference with the control group (p=0.496). Eosinophils were detected in two rabbits in group 2 and in four rabbits in group 3.

Conclusion: Captopril causes reversible inflammation in rabbit trachea, suggesting a possible mechanism for ACE inhibitor-induced cough.

Key words: Angiotensin-converting enzyme inhibitors/adverse effects; captopril/adverse effects; cough/chemically induced; inflammation; rabbits; trachea/drug effects.

Amaç: Anjiyotensin dönüştürücü enzim (ACE) inhibitörlerine bağlı öksürüğü açıklamak üzere birçok teori öne sürülmüştür; ancak, tam mekanizma bilinmemektedir. Bu çalışmada kaptoprilin tavşan trakeası üzerine olan histopatolojik etkisi araştırıldı.

Çalışma planı: Yirmi sekiz adet erkek Ankara tavşanı eşit sayıda dört gruba ayrıldı. İlk grup kontrol grubu olarak kabul edildi. Diğer üç gruba yedi gün boyunca, orofarengeal kanül yoluyla 1 mg/kg/gün oral kaptopril verildi. İntramusküler ketamin ve ksilazin hidroklorid anestezisi altında, grup 2, 3, 4'teki trakealar ilaç kesildikten sonra sırasıyla bir, yedi ve 21. günlerde çıkarıldı. Kontrol grubunun trakeaları grup 4 ile aynı günde çıkarıldı. Trakea kesitleri hematoksilen-eozin ile boyandıktan sonra ışık mikroskobu altında incelendi. Trakea dokularındaki inflamasyon kantitatif skorlama sistemi ile değerlendirildi.

Bulgular: Kaptopril kesildikten sonraki birinci gün (grup 2) ve yedinci günlerde (grup 3), kontrol grubu ile karşılaştırıldığında inflamasyonda anlamlı derecede artış saptandı (sırasıyla p=0.002 ve p=0.001); grup 2 ve 3 arasında ise anlamlı fark yoktu (p=0.872). İlacın kesilmesinden sonraki 21. günde (grup 4) ise inflamasyonun belirgin derecede gerilediği görüldü; grup 4 ile kontrol grubu arasında inflamasyon açısından anlamlı farklılık yoktu (p=0.496). Grup 2'deki iki tavşanda ve grup 3'teki dört tavşanda eozinofile rastlandı.

Sonuç: Kaptoprilin trakeada geri dönüşümlü inflamasyona yol açtığı görüldü; bu durum ACE inhibitörlerine bağlı öksürüğü açıklayıcı bir mekanizma olabilir.

Anahtar sözcükler: Anjiyotensin dönüştürücü enzim inhibitörü/yan etki; kaptopril/yan etki; öksürük/kimyasal yolla oluşan; inflamasyon; tavşan; trakea/ilaç etkisi.

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Correspondence: Dr. Ebru Ünsal. 36. Sokak, No:10/6 06550 Bahçelieveler, Ankara.
Tel: 0312 - 355 21 10 / 1043 Fax: 0312 - 355 21 35 e-mail: unsalebru73@yahoo.com

Angiotensin converting enzyme (ACE) inhibitors are widely used for the treatment of hypertension and congestive heart failure. However, ACE inhibitors have been reported to have some adverse airway reactions such as dry cough, increased airway hyperreactivity, and also bronchial obstruction in asthmatics.[1-3] Cough associated with ACE inhibitors was first reported with captopril in 1985.[4] The incidence of cough varies from 5% to 20% depending on the type of the ACE inhibitor. The cough is reversible when the drug is discontinued.[5,6]

Several hypotheses have been proposed to explain the ACE inhibitor-induced cough. Potent inflammatory mediators such as bradykinin, substance P, and prostaglandin E2 are believed to be involved in the occurrence of cough. Bradykinin is normally degraded in part by ACE and accumulate in the lung as a result of ACE inhibition, where it initiates the release of proinflammatory neuropeptides, leading to a progressively increasing inflammatory reaction in the airways.[7,8]

Experimental studies in guinea pigs showed that administration of ACE inhibitors either intradermally or intraperitoneally caused inflammatory skin reactions.[9,10] A similar inflammation in the airways was suggested to be responsible for the cough associated with ACE inhibitors.[7] However, to our knowledge, this inflammation has not been studied histopathologically in animal models. In this study, we aimed to examine the histopathological changes in rabbit trachea after captopril administration.

MATERIALS AND METHODS

Study animals. After the approval of the study by the ethics committee of our hospital, 28 male Angora rabbits, aged two years, and weighing 3 kg±200 g were divided into four groups equal in number. Prior to the study, all animals underwent one-week adaptation period in cages under normal conditions. Group 1 was accepted as the control group. Group 2, 3, and 4 received oral administration of captopril (1 mg/kg) once daily for seven days through an oropharyngeal cannula. The rabbits were anesthetized with intramuscular ketamine 50 mg/kg and xylazine hydrochloride 5 mg/kg and the tracheas were dissected on the first, seventh, and 21st days of captopril withdrawal in group 2, 3, and 4, respectively. The tracheas of the control group were dissected on the same day as group 4. After removal of tissues, the animals were sacrificed with lethal injection of pentobarbital through the ear vein.

Histopathological examination. Tracheal tissues were fixed in 10% neutral buffer formalin for histopathology. From each tracheal segment, four pieces of samples were taken, sections of 5-6 µm were obtained and stained with hematoxylin-eosin and examined under the light microscope (Nikon Labophot 2). Inflammatory cell count included lymphocytes, neutrophils, and eosinophils localized between the epithelium and cartilage. The cell count was performed on three areas of each section per high power field that had well-preserved structure, and the mean value of the cell count was calculated. Inflammation was evaluated in a blinded fashion using a quantitative scoring system:[11] 0: no inflammatory cells; 1: less than 10 inflammatory cells; 2: 10-50 inflammatory cells, 3: 50-100 inflammatory cells; 4: more than 100 inflammatory cells. The sections containing eosinophils were also determined.

Statistical analysis. For statistical analysis, the values were considered to be nonparametric. Differences in inflammation scores between the groups were analyzed by the Kruskal-Wallis test. Comparisons between two groups were made by the Mann-Whitney U-test. A p value of less than 0.05 was considered statistically significant.

RESULTS

On the first day (group 2) and seventh day (group 3) of captopril withdrawal, there was significant inflammation in the tracheas of animals compared with the control group (p=0.002, p=0.001, respectively), but

Table 1. Inflammation scores of the study groups

	Group 1	Group 2	Group 3	Group 4
Inflammation score	No. of rabbits	No. of rabbits	No. of rabbits	No. of rabbits
0	–	–	–	–
1	4	–	–	2
2	3	1	–	2
3	–	3	4	3
4	–	3	3	–

Group 1: Control group, no administration of captopril, sacrification after 21 days; Group 2, 3, and 4: Oral administration of captopril for seven days and sacrification after 1, 7, and 21 days of captopril withdrawal, respectively.

the extent of inflammation was similar in group 2 and 3 (p=0.872) (Fig. 1a, 1b). On the 21st day (group 4), inflammation reduced significantly, yielding significant differences compared to group 2 and 3 (p=0.003, p=0.002, respectively). At the end of 21 days, there was no significant difference between group 4 and the control group with respect to the degree of inflammation (p=0.496). Eosinophils were detected in two rabbits in group 2 and in four rabbits in group 3 (Fig. 1c).

DISCUSSION

ACE inhibitor-induced cough is a dry irritant cough that begins with a sense of trickling at the back of the throat.[12] Although there are several theories on the cause of ACE inhibitor-induced cough, none completely explains how ACE inhibitors produce cough. It is likely that ACE inhibitor-induced cough is related to an increased inflammatory state in the upper airways of susceptible individuals.[7,8] In this study, we demonstrated inflammation in rabbit trachea histopathologically after the administration of captopril for seven days, which might be one of the causes of this cough.

In animal studies, intraperitoneal injection of ACE inhibitors caused inflammatory skin reactions as influx of inflammatory cells (neutrophils and eosinophils) into the dermal test sites, resulting in decreases in the number of circulating eosinophils.[9,10] Cationic protein derived from eosinophils might activate the Hageman factor and contribute to the formation of bradykinin. ACE is involved in the metabolism of potent inflammatory mediators such as bradykinin and substance P.[7,13] After ACE inhibition, bradykinin releases substance P from the C-fiber terminals and both bradykinin and substance P enhance the formation of prostaglandins.[7,8,14,15] Inflammatory skin reactions are probably related to the accumulation of these proinflammatory mediators.[7] The inflammation in rabbit trachea caused by captopril might have common pathophysiological mechanisms.

In addition, bradykinin has both direct and indirect effects on the airways such as bronchoconstriction, increased mucous secretion, and bronchial edema due to capillary leakage.[15] The association between ACE inhibition and kinin dependent proinflammatory effects was reported in rat trachea previously.[16,17] We examined the histopathological changes in rabbit trachea after captopril administration. However, the dose of the drug causing the inflammation is a question. In a study by Emanueli et al.,[17] varying doses of captopril (0.5 to 5 mg/kg) were injected through the left femoral vein of mice and doses greater than 1 mg/kg were found to cause plasma extravasation and neurogenic inflammation. Based on this study, we decided to give 1 mg/kg cap-

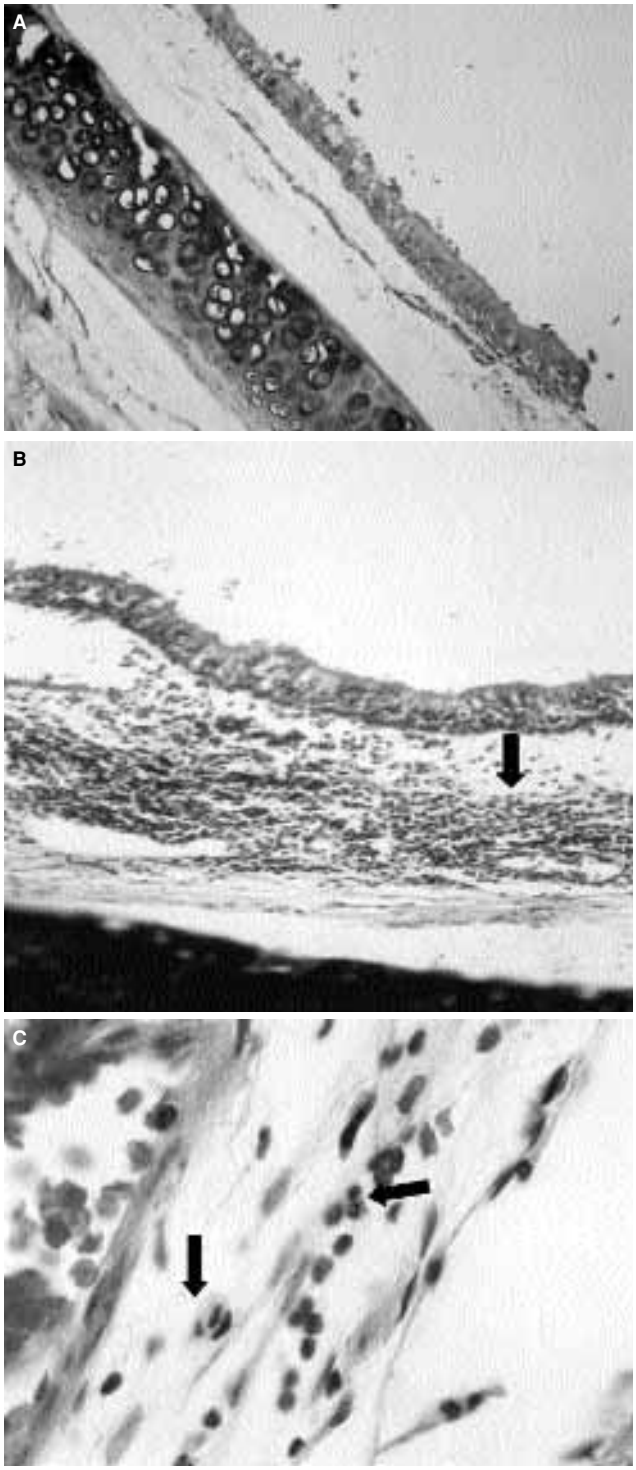

Figure 1. (A) Control rabbit trachea with inflammation score 1 (200 x H-E). **(B)** Inflammatory reactions of score 4 in the trachea of group 2 (200 x H-E). **(C)** Inflammatory reactions with eosinophils (arrows) in group 2 (1000 x H-E)

topril to the rabbits. The drug was administered by an oropharyngeal cannula to ensure that the animals received the complete dose and captopril did not go to the airways. This is a preliminary study and in further experimental studies we plan to examine the inflammation caused by varying doses of captopril and the effect of anti-inflammatory agents.

Sakamoto et al.[18] studied aminopeptidase P activity, one of the bradykinin metabolizing enzymes, in mouse trachea after administration of imidapril and enalapril. They found that aminopeptidase P activity was inhibited by enalapril, but not by imidapril, and that dry cough was higher with enalapril. They suggested that cough induced by ACE inhibitors may be related to accumulation of bradykinin in the trachea.[18] Although cessation of therapy is the only effective treatment for ACE inhibitor-induced cough, some pharmacologic agents have been shown to attenuate the cough.[19] Cromolyn sodium, theophylline, and sulindac were all studied for their anti-inflammatory effects. In some patients, these drugs were found to be effective in treating cough associated with ACE inhibitors. The possible mechanism was suggested as the inhibition of bradykinin and prostaglandin synthesis.[20] In addition, a recent experimental study showed that papaverine, a non-narcotic alkaloid, was able to decrease ACE inhibitor-induced cough.[21]

To determine if a cough is associated with the use of an ACE inhibitor, cessation is recommended for four days, after which the cough usually resolves within one to seven days.[6,20,22] In our study, the inflammatory effect of captopril continued for seven days after the drug was stopped. On the 21st day after captopril withdrawal, there was no significant difference between group 4 and the control group with respect to the degree of inflammation in rabbit trachea. This shows that inflammation in trachea caused by ACE inhibitors is reversible, but it does not disappear immediately when the drug is discontinued. We also found eosinophils in the inflammatory sections in group 2 and 3 after captopril administration. Until now, there have been only a few clinical reports about the pulmonary eosinophilic infiltration with captopril.[23,24] Schatz et al.[23] reported two patients who developed peripheral eosinophilia and new lung field infiltrates after captopril administration. Watanabe et al.[24] reported a patient with captopril-induced pulmonary infiltration with eosinophilia and concluded that this was an allergic reaction to captopril.

In conclusion, several mechanisms have been proposed to explain ACE inhibitor-induced cough. In this histopathologic study, we showed reversible inflammation in rabbit trachea which might be involved in ACE inhibitor-induced cough. As the mechanism has not been fully elucidated, further studies are needed to examine the role of this inflammation in the development of cough.

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