ERYTHROCYTE CARBONIC ANHYDRASE ACTIVITY IN SMOKERS

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SUMMARY

Carbonic anhydrase (CA) isozymes are important enzymes for body metabolism because it regulates pH in most tissues. The enzyme catalyzes the reversible hydration of CO2 to HCO₃⁻ and H⁺. Although several studies have been carried out on the inhibitors of this enzyme, those on this effect of cigarette smoking are rare.

In the present study, in vitro effects of cigarette smoke on CA were investigated. To this end, the CA activity in lyzed erythrocytes was measured in 30 smokers and 30 non-smokers. Also, in all the cases, urine and blood pH as well as K⁺ and Na⁺ in blood were investigated in order to determine side-effects that may occur based upon possible enzyme inhibition.

The carbonic anhydrase activity was lower in active smokers than non-smokers. The enzyme activities (EU) in smokers and non-smokers were measured 4350 ± 1265 and 5345 ± 1645, respectively. In terms of enzyme activity, a significant difference was found between the two groups (p<0.001). But no abnormality was determined in blood and urine pHs as well as blood Na⁺ and K⁺ levels in both groups.

Currently, the pathophysiological consequences of low CA activities in smokers remain unclarified. Though CA enzyme inhibition occurs in some of active smokers, this is likely to be compensated through other compensation mechanisms of the body. However, it is possible that this inhibition should lead to a factor predisposed to metabolic disorder, or to a disorder that can aggravate the existing disorder. In addition, it is another subject-matter of investigation why enzyme inhibition does not occur in all smokers. Further investigations are needed to answer all the questions.

Key Words: Carbonic anhydrase, cigarette smoke.

INTRODUCTION

Carbonic anhydrase (CA) has been a well characterized pH regulatory enzyme in most tissues including erythrocytes (1-4). All three soluble isozymes of CA in humans (CA I, II, and III) are monomeric, 29-kDa zinc metallo enzymes that catalyze the reversible hydration of CO2 (4,5).

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^- \]

CA I is found primarily in erythrocytes (1,5). The human CA II isozyme is widely distributed. It has been identified in erythrocytes, brain, eye, kidney, etc. Normally CA I and CA II each contribute about 50 percent of the total activity (5).

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Cigarette smoking has been implicated in the etiology of respiratory diseases, cancer, and atherosclerosis (6). Furthermore, cigarette smoking is known to affect several physiological processes including cardiovascular regulation and energy metabolism (7).

There are several different studies on the effect of cigarette smoking on enzymes (8,9). However, those carried out on its effect on CA are relatively few. The activity of this enzyme was determined to change in a variety of diseases. There is evidence that pathophysiological alterations such as diabetes mellitus and hypertension are associated with variations in CA activity (10,11). The process by various physiologic mechanisms leading to functional of CA is currently under the investigation. In addition, although enzyme inactivation has been determined in smokers, it is still unclear which substance is responsible for this situation (10).

The present study was designed to investigate the effect of cigarette smoking on CA activity in erythrocyte lysates.

**MATERIAL and METHODS**

**Subjects:** The study was conducted in 60 healthy subjects of whom 30 smokers (16 males, 14 females) and 30 were non-smokers (17 female, 13 male). The smokers group included only those having smoked for at least 20 years. The mean age was found as 49.8 years (range 41-62) in smokers and 48.8 years (range 40-60) in non-smokers. In smokers, on average 33.7 ± 13.6 pack-years was determined. The subjects were excluded if they reported a history of arthritis, cirrhosis, hypertension, diabetes, or ischemic heart disease, as they can influence CA enzyme levels.

In all cases, together with urine pH, K⁺, Na⁺ in blood, as well as HCO₃⁻ and pH in blood gases analysis were measured.

**Processing of erythrocytes:** The blood samples were centrifuged at 1500 rpm for 20 minute and the plasma and buff coat were removed. After the paced red cells were washed with NaCl (0.9%) twice, the erythrocytes were hemolyzed with cold water. The hemolysate was diluted with distilled water to the 10-fold volume, and this hemolysate was used to determine the CA activity.

**Determination of carbonic anhydrase activity:** Carbonic anhydrase activity was determined by following the hydration of CO₂ according to the method of Wilbur and Anderson (12). The enzyme unit was calculated according to the formula: 1 U = (t₀ - tₑ)/tₑ , where t₀ and tₑ are the time (sec) needed for the pH change without and with enzyme reactions, respectively.

**Statistical analysis:** For statistical analysis, Student's t test for independent samples was employed.

**RESULTS**

Carbonic anhydrase activities (EU) are shown in table 1. Smokers had significantly lower CA activities in their erythrocyte than non-smokers (p<0.001). Furthermore, 18% of the smokers was detected CA inhibition. But no
abnormality was determined in blood and urine pHs as well as blood Na\(^+\) and K\(^+\) levels in both groups.

**Table 1. Carbonic anhydrase activities (EU)**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Smokers (n=30)</th>
<th>Non-smokers (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4350</td>
<td>5345</td>
</tr>
<tr>
<td>Range</td>
<td>2775-5250</td>
<td>3575-7850</td>
</tr>
<tr>
<td>SD</td>
<td>1265</td>
<td>1645</td>
</tr>
</tbody>
</table>

Enzyme activities detected in all subjects is shown figure 1.

**Figure 1.** Enzyme activities determined in all cases.

**DISCUSSION**

In the study, the erythrocyte CA activities were found lower in healthy smokers than in the control persons. We conclude that cigarette smoking may diminish CA activity.

It is generally recognized that CA controls the bulk of carbon dioxide exchange between the blood and tissues as well as the regulation of proton and other ion movements between cells and extracellular fluids (1,3,4). There is vast literature on the chemistry and physiology of CA, with the mechanism of action of sulfonamides and inorganic anions playing an important role (4,13-15). Acetazolamide is the most common drug in this class of therapeutic agents (16). Several other factors including physical exercise and thyroid hormone have also been reported to influence CA activity. Tokuda et al described an association of increased erythrocyte CA activity with repeated strenuous
aerobic exercise. Since CA plays a major role in CO₂ removal, Tokuda et al. attributed increased erythrocyte CA activity to adaptation to a recurrently affected acid-base equilibrium (10). Inhibition of carbonic anhydrase causes wasting of Na⁺, K⁺, and HCO₃⁻ in the proximal tubules and represents a pharmacologically induced proximal renal tubular acidosis (RTA) (16). In proximal RTA, urine pH becomes <5.5. However, we did not find urine pH low in any cases, including primarily smokers. This indicates that acidosis table that is likely to develop, depending upon the enzyme inhibition that cigarette smoking causes, is compensated through other tampon mechanisms. To CA inhibition that cigarette smoking causes can be seen as a state that can be predisposed to a metabolic disorder in patients in the late stages of chronic obstructive pulmonary disease, which occurs depending upon cigarette, or that can aggravate the existing disorder.

Tobacco smoke contains large numbers of organic and inorganic compounds (17). From inhaled cigarette smoke, a broad variety of these substances is absorbed. Out of these, many could be taken into consideration to explain an effect on CA activity. One of the best-known constituents of cigarette smoke is nicotine. Nicotine as a respiratory stimulant forces CO₂ elimination. So the decrease in CA activity in smokers could be caused by adaptation to chronic influences on the acid-base balance, too (10). Furthermore, nicotine induces a general sympathetic activation with elevated catecholamine levels (7). Maybe, recurrently elevated catecholamine levels can affect CA activity. However, alteration may occur in CA activation as a complication of cigarette smoking at both lower doses and early periods. Because, Abel et al. found in their study that in cases of 25 years old who smoked at least one cigarette a day, CA activity was significantly lower with respect to control group (10). Therefore, CA inhibition is a complication that develop in all smokers. After inhalation, nicotine, because of its lipid solubility, is quickly absorbed across the alveolar surface into pulmonary capillary blood, causing a rapid increase in its levels in the arterial circulation (17). We determined CA inhibition in 18 % of our cases who were smoking.

Although some plausible explanations for the reported effects of smoking on CA can be brought forward, it remains unclear whether nicotine or other components of cigarette smoke are responsible. The mechanisms underlying the decrease in CA activity need further investigation (10).

Currently, the pathophysiological consequences of low CA activities in smokers remain unclarified. The influence of smoking habits on erythrocyte CA activity may be important for designing future studies on carbonic anhydrase. Also, prospective disorders that can develop in situations where this enzyme inhibition is determined should be investigated in detail. Moreover, it is another subject of investigation for the inhibition of this enzyme not to occur.

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ÖZET

SİGARA İÇEN OLGULARDA ERİTROSİT KARBONİK ANHİDRAZ AKTİVİTESİ

Karbonik anhidraz (CA) isozimleri bir çok dokuda pH’nin düzenlenmesinde rol oynadıklarından vücud metabolizması için önemli enzimlerdir. Enzim, CO₂’nin HCO₃⁻ ve H⁺’e reversibl hidrasyonunu katalize eder. Bu enzimin inhibitori ile ilgili çalışmalar yapılmış olmasına karşın sigaranın bu etkisi ile ilgili çalışmalar son derece azdır.


Aktif sigara içcilerinde CA aktivitesi, sigara içmemenin düşündü. Enzim aktivitesi (EU) sigara içcilerinde 4350 1265, içmeyenlerde ise 5345 1645 olarak hesaplandı. Her iki grup arasında enzim aktivitesi açısından anlamlı farklık saptandı (p<0.001). Ancak her iki grupta da idrar ve kan pH’ların ve kan K⁺ ve Na⁺ değerlerinde anormallik tespit edildi.


Anahtar Kelimeler: Karbonik anhidraz, sigara dumanı.

REFERENCES


