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Research Article

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Effect of Chronic Smoking on Mouse Corpus Cavernosum and Protector Role of Alpha-linolenic Acid

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Abstract Studies have shown the antioxidant and anti-inflammatory activity of alpha-linolenic acid, one of the omega 3 fatty acids found abundantly in linseed oil. Cigarette smoke causes impotence as it contains many harmful components and increases oxidative stress. Therefore, we planned to investigate whether or not alpha-linolenic acid has protective effect against impotence due to cigarette smoke exposure. For this purpose mice were given cigarette smoke and 200 mg / kg alpha-linolenic acid gavage for 8 weeks And phospholipase A2, inducible nitric oxide synthase (iNOS) and endothelial nitric oxide synthase (eNOS) enzymes in corpus cavernosum tissues were analyzed by ELISA. Application of cigarette smoke to the mice reduced the enzyme eNOS while boosting corpus cavernosum cyclooxygenase-2 (COX-2), phospholipase A2 and iNOS. Administration of alpha-linolenic acid to mice exposed to cigarette smoke refused the increase in COX-2, phospholipase A2 and iNOS enzymes and caused an increase in eNOS enzyme. In conclusion, our study has shown that smoking is the cause of impotence due to the increase of inflammatory mediators and the reduction of eNOS enzyme responsible for erection, and that the use of alpha-linolenic acid with smoking is beneficial against these effects.

Keywords cigarette smoke, corpus cavernosum, alpha-linolenic acid, cyclooxygenase-2, phospholipase A2, iNOS and eNOS

Introduction

Smoking is one of the important health problems of today. Exposure to cigarette smoke affects many organs, especially the lungs and the cardiovascular system, in the negative, and can cause damage and dysfunction in the involved organs. Smoking is a major risk for cardiovascular diseases. Epidemiologic studies have shown that cigarette smoking is one of the important causes of hypertension and endothelial dysfunction, leading to thrombosis, atherosclerosis, myocardial infarction, vascular graft failure and death from coronary artery disease [1-4]. Smoking reduces the combined nitrite, nitrate and antioxidant concentrations in the plasma [5]. Cigarette smoke exposure reduces NO production by affecting the L-arginine-NO synthase pathway in endothelial cells. It has also been reported in studies conducted to increase the oxidative stress. In addition, it decreases L-arginine transport and nitric oxide synthase expression and activity [6]. Prolonged cigarette smoke exposure irreversibly disrupts the structure of the mouse carotid artery and reduces its elasticity [7]. Smoking increases the risk of chronic obstructive pulmonary disease. Causing a decrease in vitamin A, leading to the development of amphizia, a disease caused by the destruction of the parenchyma cells of the liver and the decrease in elasticity [8]. Smoking increases oxidative stress by increasing free radicals. Increased oxidative stress contributes to endothelial dysfunction by inactivating NO and causes decreased blood flow in normal coronary vessels [9]. Alpha-linolenic acid, which is high in linseed oil, is a protective agent for heart and cardiovascular system [10,11]. It is the precursor of docosahexaenoic acid (DHA) and eicosapentanoic acid (EPA) which are incorporated into the arachidonate structure [12]. It has been shown that

blood pressure falls in spontaneously hypertensive rats fed with alpha-linolenic acid diet [13]. It has also been reported that japonese who had alpha-linolenic acid diets with high, medium and normal blood pressure shows lower systolic and diastolic blood pressures [14]. Alfa linoleic acid has antioxidant activity. Reducing oxidative stress also contributes to the prevention of inflammation [15]. Alfa-linolenic acid also reduces the inflammation caused by lipopolysaccharide(LPS). Alpha-linolenic acid also inhibits nuclear factor kappa-B(NF-kB) translocation and phosphorylation of mitogen-activated protein kinase (MAPK). This inhibition reduces the expression of inflammatory factors such as iNOS, COX-2, TNF-alpha [16]. In addition, omega-3 fatty acids are involved in the synthesis of prostaglandins [17]. Reduce the risk of cardiovascular diseases [18-20]. It has also been shown to regulate the immune system [21]. Prostaglandins are responsible for regulating body temperature, inflammation, pain, circulatory system, excretion system and digestive system. However, it is also important for blood clotting and synthesis of some hormones [22].

Materials and Methods

This study was approved by animal experiments local ethics committee of Cukurova University. An 8-week-old Balb / c albino male mouse weighing 30 grams were used from the Cukurova University Medical Sciences Experimental Research and Application Center. The mice were housed according to standard laboratory conditions and ethical guidelines. Mice were divided into three groups which are: Control (N=10), Applied With Smoke (N=10) and applied with Alpha-Linolenic Acid Along With Smoke(N=10). Alpha-linolenic acid was administered by oral gavage once daily for 8 weeks at a dose of 200 mg / kg. Mice to be smoked were placed in the flexographic room (100 cm long, 60 cm wide and 80 cm high) with smoke inlet region on one of the two opposite sides and the air outlet region on the other side. The combustion chamber was installed in the smoke inlet area and the cigarettes (2 units) connected to the assembly were burned and smoke flow was generated from the smoke inlet area to the air outlet area by means of the vacuum pump in the apparatus. The room was allowed to smoke for 10 minutes after each cigarette. At the end of this period, the room was allowed to remain smoke-free for 30 minutes. This cycle was applied until the end of 20 cigarettes. This practice was continued every day for 8 weeks. The same experimental conditions were also provided for the smokeless control groups and the plexiglass room was provided with air aspiration instead of smoke. At the end of the protocol described above, mice were killed by cervical dislocation. The corpus cavernosum tissues of the mice were isolated and stored in the eppendorf by freezing at -20 ° C for use in quantitative assay experiments.

Quantitative Assays

Tissue Homogenization

3ml RIPA (Radio-Immunoprecipitation Assay) buffer per gram, 30µl PMSF (fenylmetanesulfonilfluoride), 30µl sodium vanadate, 30µl protease inhibitor were added to the frozen tissues at -20 ° C in Ependorfand homogenates were obtained by breaking down the tissues on ice with an ultrasonic shredding device. The homogenates were centrifuged at 10,000 RPM for 10 minutes and the supernatants were removed and the lower precipitates (pellets) discarded.

Protein Quantification

Protein quantification of homogenized tissues was done by the Bradford method.Prepared as standard at 1, 2, 3, 5, 7, 8, 10 (µg/ml) concentrations by using bovine serum albumin (1µg/ml), then taking 10 µl of each sample and completed 100 µl by adding distilled water. After adding 1 ml of Bradford solution onto the standard and samples, mixing with vortex then absorbance quantities were measured at 595 nanometer in the spectrophotometer. Protein quantity was determined (µg / µl) according to the standard curve plotted in the Prism program.

ELISA (Enzyme Linked Immunosorbent Assay) Test

Expressions of cyclooxygenase-2, phospholipase A2, iNOS and eNOS enzymes were examined by ELISA test. The ELISA protocol is variable for each kit.

Evaluation of Results

The relaxation responses of the tissues were expressed as a percentage of contractions. Standard errors was Shown with (Mean ± SEM). Graphpad Prism (CA, USA) program was used for drawing and statistical analysis of the graphs. One way (ANOVA) for statistical comparisons and Bonferroni for post hoc test were used. P values less than 0.05 were considered significant.

Results

ELISA Phospholipase A2 Enzyme Quantification Assay

While cigarette smoke exposure caused an increase in phospholipase A2 enzyme in the corpus cavernosum, alpha-linolenic acid administration with cigarette smoke significantly reversed this increase (Figure 1).

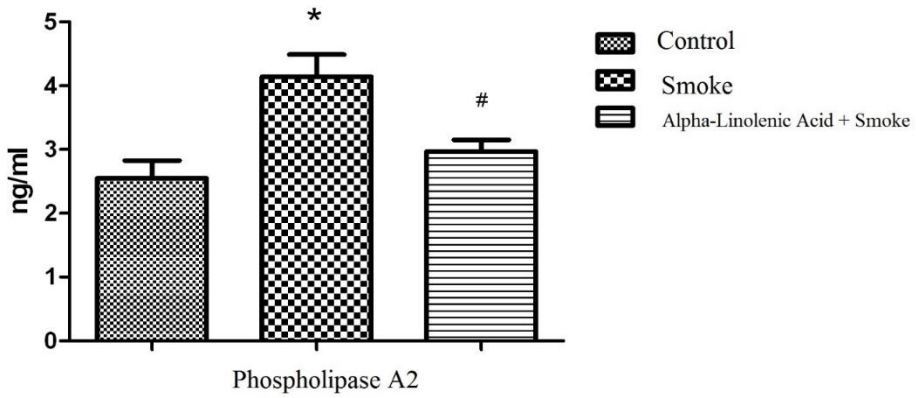

Figure 1: Effect of cigarette smoke exposure on phospholipase A2 enzymes in corpus cavernosum of rats (n = 6). Statistical analysis: ANOVA. Post hoc: Bonferroni. (: compared to control P <0.05. #: according to smoke applied group P <0.05).*

ELISA Cyclooxygenase-2 Enzyme Quantitation

Exposure to cigarette smoke caused an increase in the cyclooxygenase-2 enzyme in the corpus cavernosum, whereas administration of alpha-linolenic acid with cigarette smoke significantly reduced this increase (Figure 2).

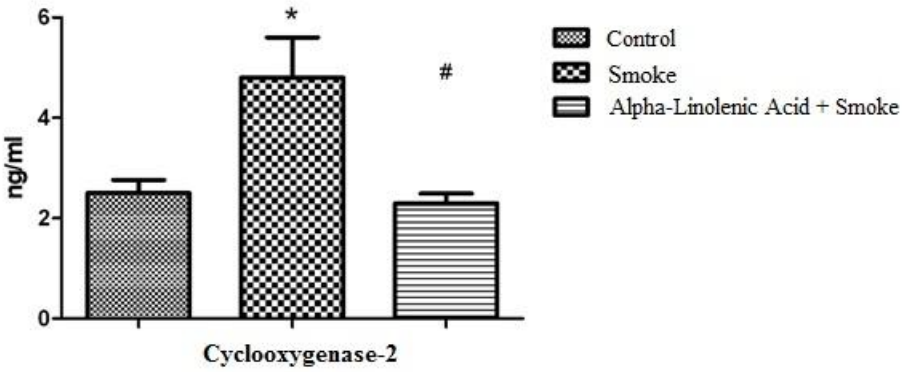

Figure 2: Effect of cigarette smoke exposure on the cyclooxygenase-2 enzyme in the corpus cavernosum of rats (n = 6). Statistical analysis: ANOVA. Post hoc: Bonferroni. (: compared to control P <0.05. #:according to smoke applied group P <0.05).*

ELISA iNOS Enzyme Quantification

Cigarette smoke Exposure caused an increase in iNOS enzyme in corpus cavernosum, while administration of alpha-linolenic acid with cigarette smoke significantly reduced this increase (Figure 3).

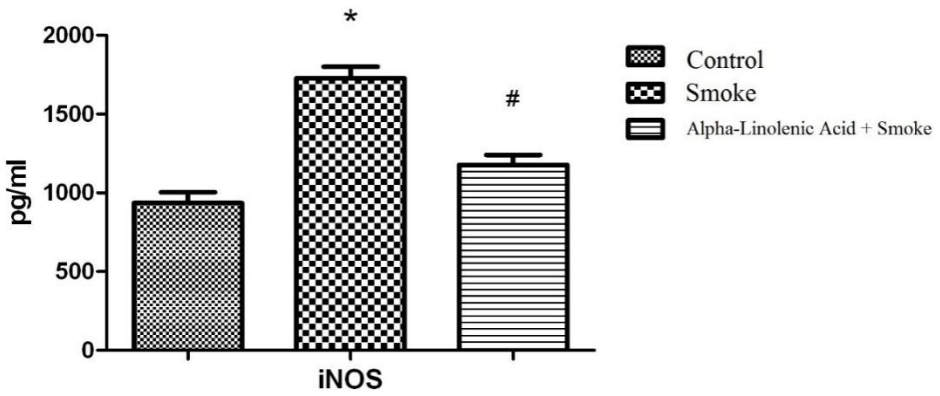

Figure 3: Effect of cigarette smoke exposure on the iNOS enzyme in corpus cavernosum (n = 6). Statistical analysis: ANOVA. Post hoc: Bonferroni. ***(*:****compared to control P <0.05 #: according to smoke applied group P <0.05).*

ELISA eNOS Enzyme Quantification Assay

Application of alpha-linolenic acid with cigarette smoke significantly increased the amount of eNOS, while exposure to cigarette smoke caused the eNOS enzyme in the corpus cavernosum to decrease (Figure 4).

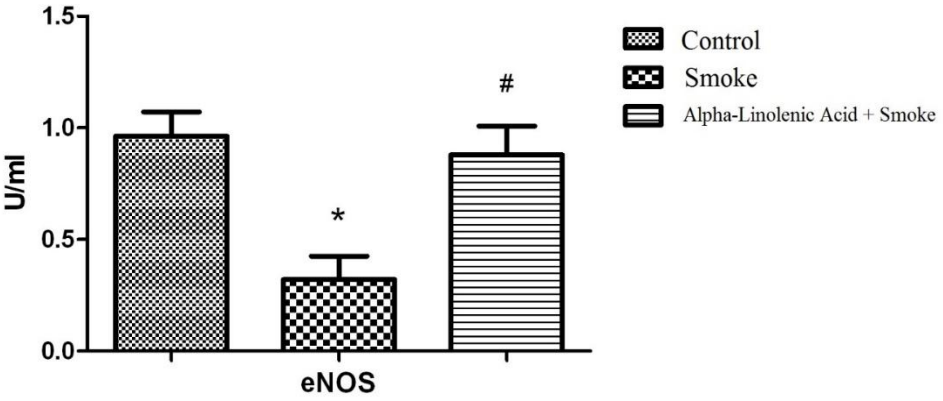

Figure 4: Effect of cigarette smoke exposure on eNOS enzymes in corpus cavernosum of mouse (n = 6). Statistical analysis: ANOVA. Post hoc: Bonferroni. (: compared to control P <0.05 #: according to smoke applied group P <0.05).*

Discussion

In our study, we evaluated phospholipase A2 and cyclooxygenase-2 enzymes, which act as biological mediators in the inflammatory response, and eNOS enzyme, the NO producing enzyme responsible for the expansion of the corpus cavernosum tissue. Cigarette smoke has been shown in other studies that increase the enzymes of cyclooxygenase-2 and phospholipase A2, which play important roles in inflammatory mechanisms [23-24]. In our study, similarly, cigarette smoke caused an increase in the amount of cyclooxygenase-2 and phospholipase A2 enzymes, while alpha-linolenic acid, which showed anti-inflammatory properties, significantly decreased these increases. In one study, it was shown that cigarette smoke increased the activity of NADPH oxidase enzyme and increased the production of intracellular superoxide radicals, which increased the level of cytosolic phospholipase A2 enzyme.This increase has been shown to be due to the MAPKs pathway, NF-KB, and AP-1 factors that activate proinflammatory gene transcription [25]. In another study, similarly, cigarette smoke increased the cyclooxygenase-2 enzyme. This increase has been shown to be due to increased NF- [kappa] B and p300 inflammatory transcription factors by activation of the PKC / c-Src / EGFR and PDGFR / PI3K / Akt PDGFR / PI3K / Akt signaling pathways

[23]. Phospholipase A2 is located at the top step in the inflammatory mechanism and causes the synthesis of arachidonic acid, the substrate of cyclooxygenase-2 enzyme from the membrane phospholipids. The inhibition of this step with alpha-linolenic acid indicates that it will initially inhibit inflammation. Studies have shown that cigarette smoke increases peroxynitrite production due to increased iNOS activity because of oxidative stress [26]. Furthermore, activity of INOS and peroxynitritin have been shown to cause an increase in COX-2 activity. Reduction of oxidative stress due to the antioxidant property of alpha-linolenic acid may have caused a decrease in iNOS and COX-2 enzymes. The fact that cigarette smoke causes down regulation of the eNOS enzyme may reduce the erection because it will be reflected in the production of NO responsible for erosion. Because alpha-linolenic acid improves this inhibition of enzyme and it will protect over against the impulse caused by the cigarette. Cigarette smoke causes an increase in the amount of cyclooxygenase-2, phospholipase A2 and iNOS enzymes, resulting in damage to the corpus cavernosum due to increased migration of monocytes and macrophages by the expression of cytokines, chemokines and adhesion molecules.

Conclusion

Our study has shown that inhibition of these enzymes with alpha-linolenic acid inhibits the migration of monocytes and macrophages and protects against possible damage to the corpus cavernosum. As a result, it has been shown that with the use of cigarettes, the alpha-linolenic acid diet may help to reduce the negative effects of cigarette smoking.

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