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EFFECTS OF CIGARETTE SMOKING ON THE ULTRASTRUCTURE OF RAT AORTIC ENDOTHELIUM

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SUMMARY

In this study, rats in experimental group were exposed to smoke-air mixture (1/9 volume) two hours/day, consecutively 60 days. Control animals exposed to room air. It was observed at the 60th day that microvilli-like structures formed on the apical surface of endothelium cells of the rats exposed to cigarette smoke. At this stage, cells had polygonal structure. However, it was established that small particles accumulated on the surface of the endothelium cells and endocytosis increased. It was also observed that subendothelial layer became irregular.

Key words: Endothelium, cigarette smoke, ultrastructure

SIÇAN AORTİK ENDOTELYUMUNUN İNCE YAPISI ÜZERİNE SİGARA DUMANININ ETKİSİ

ÖZET

Bu çalışmada deney grubundaki farelere sigara dumanı-hava karışımı (1/9 oranında) 60 gün boyunca günde iki saat verildi. Kontrol sıçanlar ise oda havasına maruz bırakıldı. Sigara dumanına maruz bırakılan sıçanların endotelyum hücrelerinin apikal yüzeyinde microvillus benzeri yapıların meydana geldiği gözlenmiştir. Bu safhada hücreler poligonal bir yapıya dönüşmüşlerdir. Bununla beraber endotel hücrelerinin yüzeyinde küçük partiküllerin biriktiği ve endositozun arttığı tespit edilmiştir. Subendotelyal tabakanın da düzensizleştiği gözlenmiştir.

Anahtar kelimeler: Endotelyum, sigara dumanı, ultrastrüktür

INTRODUCTION

Epidemiological studies have shown that there is a high order of correlation between cigarette smoking and cardiovascular disease, especially atherosclerosis (1-3). While it is known that cigarette smoking is associated with an increase in atherosclerosis the mechanisms by which this is brought about are not known (4).

Injury to the cell layer lining the lumen of arteries, the arterial endothelium is fundamental in the initiation and progression of arterial lesions such as intimal hyperplasia and atherosclerosis (5). Such pathological changes in arterial intima are clinically associated with myocardial infarction, arterial aneurysms, stroke and peripheral vascular diseases (6). Cigarette smoking has been shown to result in endothelial cell alteration in animal studies (7, 8) and these results are supported by available human data (9, 10). Recently, subendothelial damage has been described in the aortas of rats exposed to cigarette smoke

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(11). Evidence concerning the specific components in tobacco smoke that cause vessel wall damage is scanty, although oral consumption of nicotine is known to result in damage to the aortic endothelium of rabbits (12).

The aim of our studies was to investigate the effects of smoke exposure on aortic endothelium in rat with transmission electron microscope.

MATERIAL AND METHODS

Animals

Male Wistar rats (weighing 200-250 g) were randomly divided into the experimental and control groups. All rats were housed in stainless steel wire cages except the period of smoke exposure and were fed on standard rat pellets and tap water ad libitum. Twelve rats in experimental group were exposed to cigarette smoke by using continuous exposure system two hours/day, seven days/week for 60 days. Control rats (n:15) were restrained in an identical system but only exposed to room air.

Smoke generation and exposure

Smoke exposure system consist of three glass chambers, pumps and fans. Centrifugal fans were attached to all chambers of the smoking machine for obtaining smoke-air mixture homogeneously. One pump was used to push air through the burning cigarette into the rate of 5.2 L/minute. By removing of the filter, each cigarette was smoked approximately for 10 minutes.

Preparation of the tissue samples

For electron microscopic examination of the tissues, primer fixation was made in 2.5% glutaraldehyde in sodium phosphate buffer, pH 7.4 for three hours at 4°C. Materials were washed with sodium phosphate buffer pH 7.4 for two hours at 4°C and post-fixed in 1% O_sO_4 and in sodium phosphate buffer pH 7.4 for one hour at 4°C. Tissue samples were washed with same buffer for two hours at 4°C and dehydrated in gradual alcohol and propylene oxide. Tissues were embedded Araldite CY212. Thin sections were prepared by Reichert OM U3 ultramicrotome, transferred to 300 mesh grids stained with 2% uranyl acetate and lead citrate. Following these procedures, the sections were viewed and photographed on a Jeol 100 CX II electron microscope at 80 kW.

RESULTS AND DISCUSSION

In the rats of control group, the endothelium in the inner layer of aorta was found to be occurred as flat cells. The nucleus was found to be placed in the center of these cells as adapted to the shape of these cells. Mitochondria and endoplasmic reticulum were seen in the cytoplasm of endothelial cells. Besides, there were small vesicles in the apical cytoplasm of endothelial cells. Subendothelial area are made up of from connective tissue containing collagen and elastic fibers (Fig 1).

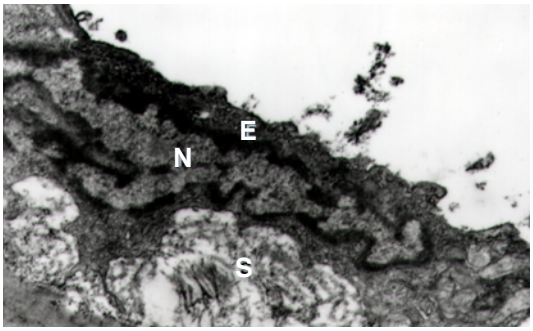

Figure 1. Electron micrograph of the aortic endothelium of the control group. E: Endothelial cell, N: Nucleus, S: Subendothelial area, X17500.

At the 60th day of exposure to cigarette smoke, certain changes in the cytological structures of endothelial cells were also noticeable. In the luminal surface of endothelial cells, numerous number of microvillus-like structures were observed. Some of the cells which are generally in the shape of flat were seen to be appear as polygonal (Fig 2). An increase in the number of plasmalemmal vesicles in the endothelial cells were also noticed (Fig 3). Besides an accumulation of small particles on the luminal surface was detected. Formation of oedema on the subendothelial layer was seen (Fig 4). In addition, the atrophy formation in some endothelium cells, swelling of mitochondria and irregular

structure of luminal layer of aorta were noticed.

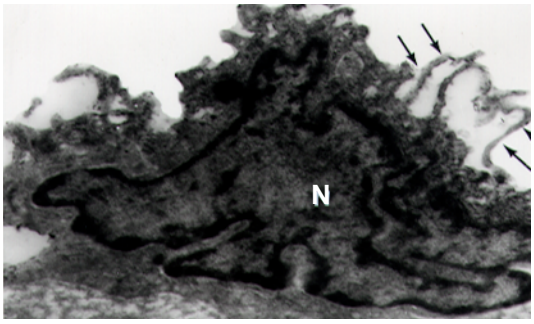

Figure 2. Polygonal cells containing microvillus-like structure (→) at the 60th day of smoke-exposure. N: Nucleus, X17500.

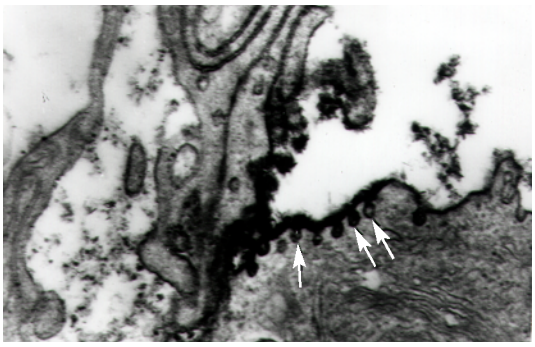

Figure 3. Increase in the plasmalemmal vesicle (→) of the aortic endothelium at the 60th day of smoke-exposure, X40000.

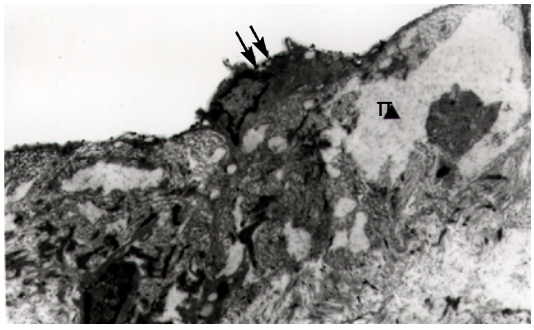

Figure 4. Oedema (π) of the subendothelial layer of aorta at the 60th day of smoke-exposure, (→): small particle, X7500.

The role of cigarette smoking as a risk factor for development of cardiovascular disease has been recognized since the large epidemiological studies of the 1950's (13). Autopsy studies identified atherosclerosis as the basic disease process behind the clinical cardiovascular problems associated with cigarette smoking in earlier studies. Subsequent research elucidated the cellular events involved in the development of atherosclerosis and identified 'injury to arterial endothelium' as a fundamental step in the pathogenesis of these lesions (14). Atherosclerosis and its complications are the major causes of mortality in Japan. Hypercholesterolemia is the most common risk factor associated with atherosclerosis in modern industrialized countries. The lesion in man is characterized by accumulation of lipids in and around the cells of the intima and is associated with cellular and fibrous proliferation resulting in a subsequent narrowing of the vessel lumen (15, 16).

Atherosclerosis is one of the illnesses caused by smoking. It was also stated that smoking causes infertility by decreasing sperm quality and number (17). Besides, any kind of health problem happening in active smokers has been based on smoking.

Experimental models have shown that endothelial injury is ultrastructurally characterized by endothelial cells swelling, cytoplasmic vacuolation, increased irregularity of the luminal surface, mitochondrial swelling and subendothelial oedema (8, 14).

The results obtained in this study are in good harmony with the others (8,14-16). In this study, at the 60th day of exposure, endothelium cells were found to be mostly defected when aorta of experiment group was examined. The increase in the number of small particles on endothelial cells of rats attracted attention and this may possible cause the formation of microvillus-like structures in the endothelial cells and increase in the number of plasmalemmal vesicles.

The defect in subendothelial layer was also seen to be took place. In this study,

endothelial cells and subendothelial layer were examined cytopathologically. The other layers of aorta were not examined. However, based on our results and the results obtained from other studies (8,14-16) it may be possible to conclude that pathological changes occur in the other layers.

REFERENCES

1. Murphy EA, Mustard JF. Tobacco and thrombosis. Am J Pub Health 1966; 56: 1061-1073.
2. Dawber TR, Kannel WB, Revotskie N, Stokes J, Kagan A, Gordon T. Some factors associated with the development of coronary heart disease: Six years follow-up experience in the Framingham study. Am J Pub Health. 1959; 49: 1349-1356.
3. Spain DM, Bradess VA. Sudden death from coronary heart disease: Survival time, frequency of thrombi and cigarette smoking. Chest 1970; 58: 107-110.
4. Strong JP, Richards ML. Cigarette smoking and atherosclerosis in autopsied men. Atherosclerosis. 1976; 23: 451-476.
5. Ross R Glomset JA. The pathogenesis of atherosclerosis. N. Eng. J. Med., 1976; 295: 420.
6. Report of the surgeon general smoking and Health, US Department of Health Education and Welfare, Cardiovascular Disease. 1979; pp 4-77.
7. Bazin M, Turcotte H, Lagace R, Boutet M. Effect cardiovasculiares de la fumee de cigarette chez le rat. Rev. Can. Biol. 1981; 40: 263-276.
8. Pittilo RM, Bull HA, Gulati S, Rowles PM, Blow CM , Machin SJ, Woolf N. Nicotine and cigarette smoking: Effects on the ultrastructure of aortic endothelium. Int J Exp Path 1990; 71: 573-586.
9. Assusmen I. Chromatin changes of endothelial cells in umbilical arteries in smokers. Clin Cardiol 1982; 5: 653-656.
10. Asmussen I. Ultrastructure of the umbilical arteries from new-born delivered at term by a mother who smoked 50 cigarettes per day. Acta Path Scand 1982; 90: 397-404.
11. Boutet M, Bazin M, Turcotte H, Lagace R. Effects of cigarette smoke on rat thoracic aorta. Artery. 1980; 7: 56-72.
12. Booyse FM, Osikowicz G, Quarfoot AJ. Effects of chronic oral consumption of nicotine on the rabbit aortic endothelium. Am J Pathol 1981; 2: 229-238.
13. Royal College of Physicians, Smoking and Health Now. A new report and summary on smoking and its effects on health. Pitman, London, 1971; pp 9-148.
14. Zimmerman M, McGeachie J. The effect of nicotine on aortic endothelium: A quantitative ultrastructural study. Atherosclerosis 1987; 63: 33-41.
15. Watanabe T, Hirata M. Atherosclerosis. Sogo Rinsho 1984; 33: 647-655.
16. Watanabe T, Tokunaga O, Fan J. Atherosclerosis and Macrophages. Acta Pathologica Japonica. 1989; 39 (8): 473-486.
17. Yardımcı S, Atan A, Taştan H, Kalender Y, Kalender S, Sunguroğlu K, Avunduk MC. Effects of cigarette smoke exposure on spermatogenesis in rat. Journal of Ankara Medical School. 1996; 18 (4): 181-186.