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Microscopy of Rat Thoracic Aorta in Chronic Ethanol Intake*[†]

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Abstract: The aim of this study was to determine the histological changes of the thoracic aorta in rats fed on alcohol for a period of 24 weeks.

Twenty male Wistar rats were divided into two groups. The ethanol group (n=10) was fed on a modified liquid diet (MLD) containing 7.2% ethanol for 24 weeks. The control group (n=10) was fed on MLD without ethanol throughout the experiment. At the end of the 24 weeks, the carotid arteries of the rats were cannulated and their blood pressures were measured. Ethanol levels measured by using the NAD⁺/NADH enzyme-spectrophotometric method. Rats were killed by decapitation. 3 mm long segments of thoracic aortae were fixed in 10% neutral buffered formalin and investigated by light microscope, following routine tissue processing and staining.

In the ethanol group, rats had increased systolic and diastolic blood pressures. In the tunica intima, there was no evidence of any alcohol-induced effect that could indicate disruption, but some of the rats showed focal changes in their tunica media. The elastic lamellae of the media were degenerated and fragmented. The aorta wall was thicker in these areas. The tunica adventitia was normal and showed no differences from that of the controls.

Our results revealed that chronic alcohol intake degenerates media architecture in the rat thoracic aorta, causes fragmentation of elastic lamellae and destroys the lamellar unit.

Key Words: thoracic aorta, ethanol, rat, light microscopy

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Introduction

Chronic alcohol ingestion has been found to affect many systems, including the cardiovascular system, in humans. Hypertension, coronary heart disease and congestive cardiomyopathy are reported in alcoholics (1-4).

The association between high blood pressure and chronic alcoholism has been well documented (5). Acetaldehyde, the primary product of ethanol metabolism, has been found to increase the generation of angiotensin I, which causes an increment in angiotensin II levels (2,6). Angiotensin II is known as a powerful vasoconstrictor acting directly upon blood vessels, also found to promote the biosynthesis of transforming growth factor β_1 , which stimulates extracellular matrix production. This production can lead to fibrosis (2).

As a result, ethanol intake can alter vessel histology directly by fibrosis or indirectly by the destructive effects of hypertension.

The aim of this study was to determine the histological changes of the thoracic aorta in rats fed on alcohol for a period of 24 weeks.

Materials and Methods

Ethanol Treatment

Twenty adult male Wistar rats weighing 180-250g were used. They were placed individual cages in a quiet room with controlled temperature ($21\pm 2^\circ\text{C}$) and humidity ($60\pm 5\%$) in which a 12-12 h light-dark cycle was maintained. Rats were fed on a modified liquid diet (MLD) for seven days(7). The MLD are composed of cow's milk 925ml, vitamin A 5000 IU and sucrose 17 g.

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At the end of seven days, rats were divided into two groups. MLD with 2.4% ethanol (v/v) (ethanol 95.6%, Tekel, Turkish State Monopoly) was administered to the ethanol group (n=10) for 3 days. Then the ethanol concentration was increased to 4.8% for three days and finally to 7.2% for 24 weeks. When ethanol concentration was increased, sucrose was reduced to maintain the isocaloricity of the diet. The control group (control group, n=10) was given MLD without ethanol throughout the experiment. MLD was prepared daily and administered at 11.00 hrs every day. The diet was offered in special glass bottles to prevent spillage. All rats (control and alcoholized) were maintained on the liquid regimen for 24 weeks. The animals were excellent in health throughout the treatment. At the time of killing, all animals were approximately at the same weight (control group 291.6 ± 12.3; ethanol group 281.6 ± 9.5). Every day alcohol intake was measured. At the end of the 24 weeks, the carotid arteries of the rats were cannulated and their blood pressures were measured (Harvard 50-8952) and recorded (Harvard Universal Pencoder), under urethane anesthesia (1.2-1.4g/kg). Their blood was collected to measure ethanol concentrations. Serum was assayed for ethanol levels by the NAD⁺/NADH enzyme-spectrophotometric method (8).

All experiments in this study were performed in accordance with the guidelines for animal research from the National Institutes of Health (NIH publication 85-23, revised 1985) and were approved by the Committee on Animal Research at Inonu University, Malatya.

Light microscopy

Rats were killed by decapitation. Thoracic aorta segments were fixed in 10% neutral buffered formalin solution for 24 hours. The sections were embedded in paraffin following routine tissue processing. Serial 6µm sections were stained with hematoxylin-eosin and by Orcein and Masson's trichrome stainings (9). Vascular histology was examined by Olympus BH2 photomicroscope.

Statistics

Results were expressed as arithmetic mean ± SEM. Statistical analysis of the data was performed by using Mann-Whitney U test and the differences between the groups of data were considered to be significant when p<0.05.

Results

Blood pressures

Systolic and diastolic blood pressures were higher in the ethanol group than in the control group (Table).

	Diastolic pressures	Systolic pressures	number
control group	59.5±1.89	90.5±1.74	10
ethanol group	82.0±2.38	102.5±1.70	10
Mann-Whitney U test.	p<0.05		

Table: Blood pressures of control and ethanol groups.

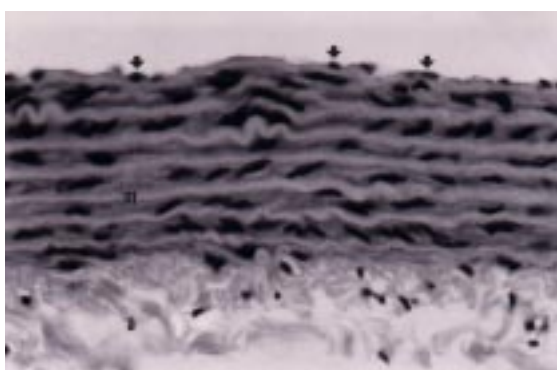


Figure 1. Thoracic aorta of the control group. Endothelial cells (arrows), tunica media (m), tunica adventitia (a). Hematoxylin eosin, X132.

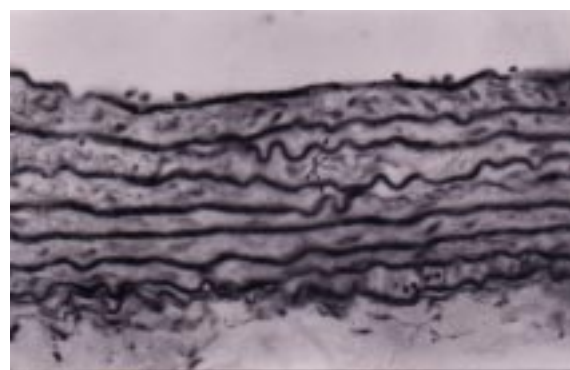


Figure 2. Thoracic aorta of the control group. Normal elastic lamellae (l) architecture. Orcein staining, X132.

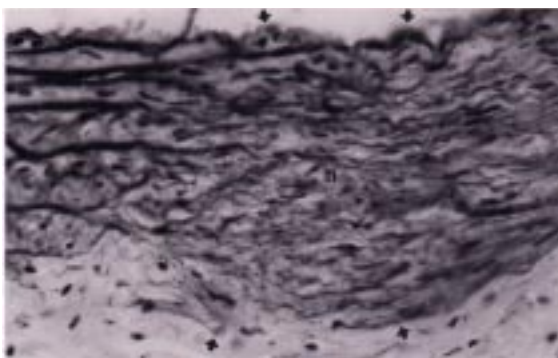


Figure 3. Thoracic aorta of the ethanol group. Tunica media (m) shows focal changes. Tunica media is thick in the affected region (arrows). Orcein staining, X132.

Ethanol consumption and blood ethanol levels

Ethanol consumption of the alcoholized rats for the individual days ranged from 13.36 ± 0.55 to 17.57 ± 0.63 g/kg/day. The mean blood ethanol level was 2.78 ± 0.24 mg/ml (n=10) in ethanol-fed rats.

Light microscopy

In the control group, the tunica intima, media and adventitia of all rat specimens (n=10) showed normal histology (Figure 1).

In the ethanol group, there was no evidence of any alcohol-induced effect that could indicate disruption of the tunica intima. However, specimens of 3 (out of 10) ethanol group rats showed focal changes in their tunica media. With Orcein staining, in these focal areas, the elastic lamellae of the media were degenerated and fragmented (Figures 2,3). The aorta wall was thicker in these areas. However, we detected no fibrosis in these focal areas by Masson's trichrome staining.

The tunica adventitia was also normal and showed no differences from controls in all sections with all staining methods (Figure 3).

Discussion

In the liver, alcohol is metabolized to acetaldehyde by three enzyme systems: alcohol dehydrogenase, microsomal enzyme oxidizing system, and catalase (2,10). Alcohol dehydrogenase has the principal activity, and it is also present in blood vessels (3). Allali-Hassani et al. (3) showed class IV alcohol dehydrogenase activity in

the arterial endothelium and mostly in the media of rat vessels. They suggested that ethanol metabolism by vascular alcohol dehydrogenase may contribute to vascular damage.

Acetaldehyde has been found to be responsible for hypertension in chronic alcoholics. Two mechanisms have been suggested to be responsible by Brecher et al. (2): increased generation of angiotensin I and excitation of transforming growth factor β_1 production which leads to extracellular matrix synthesis and fibrosis. In our study, fibrosis in the thoracic aorta was not observed.

The basic element of the aortic media is the "lamellar unit", consisting of two elastic lamellae and intervening tissue. Most lamellar units contain a single layer of smooth muscle cells that, on light microscopic examination, seem to be separated by extracellular matrix from the elastic lamellae on either side (11). In our study, prominent elastic lamellae fragmentation was found. The lamellar unit had been devastated focally. Elastic lamella fragmentation is a major microscopic finding of dissecting aneurysms, in the etiology of which hypertension plays an important role (12,13). In the present study, the ethanol group rats were hypertensive at the end of 24 weeks.

Maeda et al. (14) detected elastic lamellae breaks and fragmentation in the aortic walls of vitamin-C deficient mice. Ethanol ingestion causes nutritional deficiency in humans (1,15). Suresh et al. (16) observed that alcohol administration causes depletion in the tissue ascorbic acid content of guinea pigs. We did not assay vitamin C levels in rats, but in general, ethanol treated rats were healthy throughout the 24 weeks and their weights were close to those of the control treated rats.

In our study, the tunica intima and adventitia were found to be normal, and no signs of atherosclerosis detected. Our findings on the tunica intima were published elsewhere (17).

The effects of the chronic alcohol intake that were observed in the rat thoracic aorta were probably an indirect result of hypertension. However, the local effects of alcohol dehydrogenase in the aorta wall and vitamin C deficiency caused by chronic alcohol intake may also be important in the process. They may have additive effects.

Our results reveal that chronic alcohol intake degenerates media architecture in rat thoracic aorta, and causes fragmentation of elastic lamellae. These changes are similar to the histopathological changes seen in dissecting aneurysms in humans.

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