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Histological Changes in the Rat Thoracic Aorta after Chronic Nitric Oxide Synthase Inhibition

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Abstract: The objective of this study was to determine the morphological effects of chronic nitric oxide (NO) inhibition on the thoracic aorta of Wistar rats.

Fifteen male Wistar Albino rats were divided into three groups. Group I rats (control group, n = 5) received tap water, group II rats (n = 5) recieved 100 mg/kg L-NAME (moderate NO inhibiton) and group III rats (n = 5) received 500 mg/kg L-NAME, in drinking water (severe NO inhibition) for 15 days. At the end of 15 days, the carotid arteries of the rats were cannulated and their blood pressure was measured. The rats were sacrificed and thoracic aorta segments were fixed in 10% neutral buffered formalin solution and examined by light microscope. The blood pressure results were expressed as the arithmetic mean ± SEM. Statistical analysis of the data was performed by the Kruskal Wallis H test ($p < 0.05$). The differences between the groups were evaluated by the Mann-Whitney U test.

Group I showed normal blood pressure and histology. Groups II and III had hypertensive blood pressure and showed vascular wall thickening. Irregular luminal layers of the endothelial cell linings and increased intensity of anti-∝-SMA labeling were seen in both experimental groups.

Our results revealed that chronic NO inhibition led to hypertension and structural changes in the thoracic aorta wall in Wistar rats.

Key Words: thoracic aorta, nitric oxide, L-NAME, rat, light microscopy

Introduction

The discovery that mammalian cells generate nitric oxide (NO), a gas previously considered to be merely an atmospheric pollutant, is providing important information about many biological processes (1).

NO is formed from the aminoacid L-arginine by a family of enzymes, the NO synthases (NOS). Vascular endothelial cells synthesize and release NO, which plays an important role in the regulation of vascular tone, platelet aggregation, leukocyte adhesion and vascular smooth muscle cell proliferation (2-4).

NOS activity can be inhibited by L-arginine analogues such as N-nitro-L-arginine methyl ester (L-NAME) or N-monomethyl L-arginine (L-NMMA) (2). It was reported that chronic inhibiton of NOS with L-arginine analogue caused systemic arterial hypertension in normal rats (5) and accelerated atherosclerotic lesion, in cholesterol-fed rabbits (6). There are also some investigations indicating that chronic NO inhibition contributes to vascular structural changes (2,4). However, vascular structural and functional changes associated with the chronic inhibition of NOS are poorly understood.

This study was designed to determine whether the chronic inhibition of NO synthesis with L-NAME would cause structural changes in the aorta of Wistar rats.

Materials and Methods

Animal groups and NOS inhibition

The experiment was performed on 15 male Wistar Albino rats (average weight 250 g) purchased from the İnönü University Animal Laboratory. The rats were housed in individual cages in a quiet room with controlled temperature (21 ± 2 ºC) and humidity (60 ± 5%) in which a 12-12 h light-dark cycle (7 am to 7 pm) was maintained.

The rats were divided into three groups; group I rats (control group, n = 5) received tap water, group II rats (n = 5) received 100 mg/kg L-NAME with their drinking water for 15 days (moderate NO inhibiton) and group III rats (n = 5) rats received 500 mg/kg L-NAME in drinking water (severe NO inhibition) for 15 days.

Measurement of Blood Pressure

After 15 days of treatment a catheter was introduced into the right carotid artery of each rat for the measurement of arterial blood pressure under urethane anesthesia (1.2 g/kg, intraperitoneally). The arterial blood pressure was monitored by a Harvard model 50-8952 transducer and displayed on a Harvard Universal pen-recorder.

Light Microscopy

The rats were sacrificed and thoracic aorta segments were fixed in 10% neutral buffered formalin solution for 24 h. The sections were embedded in paraffin following routine tissue processing. Serial 6 µm sections were stained with hematoxylin eosin (H-E), Orcein, Masson's trichrome and anti-∝-smooth muscle actin methods. Stained sections were examined and photographed by an Olympus BH-2 photomicroscope.

Statistics

The results were expressed as an arithmetic mean ± SEM. The statistical analysis of the data was performed by the Kruskal Wallis H test and the data were considered to be significant when $P < 0.05$. The differences between the groups were evaluated by the Mann-Whitney U test.

Results

Blood Pressure

Systolic and diastolic blood pressure were increased in groups II and III compared with the control group (Table).

Table. Blood pressure of I (control), II (100 mg/l L-NAME) and III (500 mg/l L- NAME) groups.

	Diastolic pressure*	Systolic pressure*
I. group (n = 5)	56.00 ± 2.45[a]	94.00 ± 2.45[a]
II. group (n = 5)	91.00 ± 8.67	132.00 ± 3.39
III. group (n = 5)	89.00 ± 7.67	138.00 ± 5.83

*p < 0,05, Kruskall-Wallis
[a]p < 0,01, Mann-Whitney U

There was no significant difference in mean blood pressure between groups II and III.

Light microscopy

Control group (group I)

The tunica intima, media and adventitia of the control group rat specimens showed normal histology (Figure 1). The intima of the aorta was composed of a continuous layer of endothelial cells (Figure 2). The tunica media was observed by numerous distinct elastic laminae, which

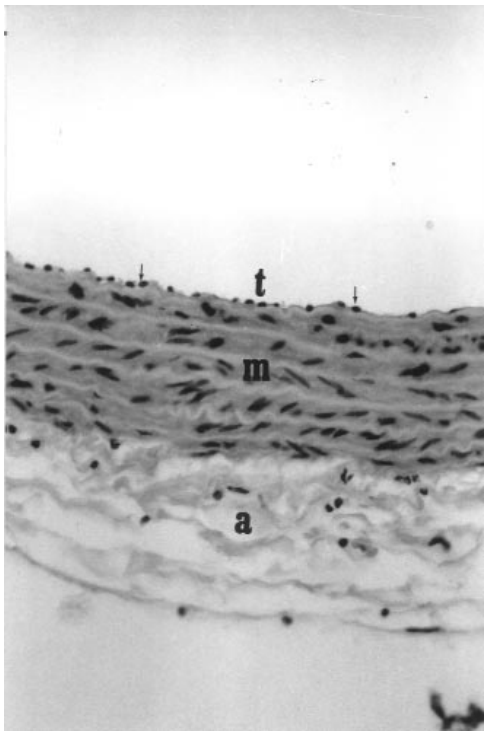

Figure 1. Control group. Tunica intima (t), tunica media (m) and tunica adventitia (a) appear normal. H-E X 66.

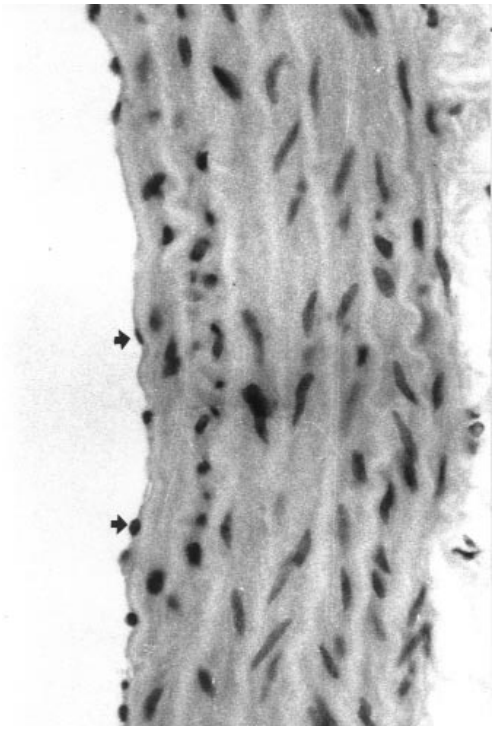

Figure 2. Control group. Intima is composed of a single layer of endothelial cells (arrows). H-E X 132.

were wavy and arranged concentrically, by Orcein staining (Figure 3). With anti-α-smooth muscle actin (α-SMA) labeling, smooth muscle cells were seen in the interspaces between the concentric lamellae (Figure 4).

Experiment groups (groups II and III)

The experimental groups showed vascular wall thickening. Focal tunica intima thickening and diffuse tunica media thickening were observed. This thickening was more prominent in group III (Figures 5 and 6).

Irregular luminal layers of endothelial cell linings were noted in both groups, particularly in group III (Figure 7). The most prominent lesion of the tunica intima was characterized by focal thickening in both L-NAME groups. In these areas connective tissue cells were seen but seldom anti-α-SMA (+) cells (Figure 8).

The media consisting of series of concentrically arranged elastic laminae were visible in both L-NAME groups. When compared with the control group (Figure 3), the number of elastic lemellae were found to increase parallel to the wall thickening (Figures 9 and 10).

The media of the thoracic aortae from groups II and III showed an increased intensity of anti-∝-SMA labeling (Figure 11) when compared with the control group (Figure 4). The adventitia showed normal histology in both experimental groups.

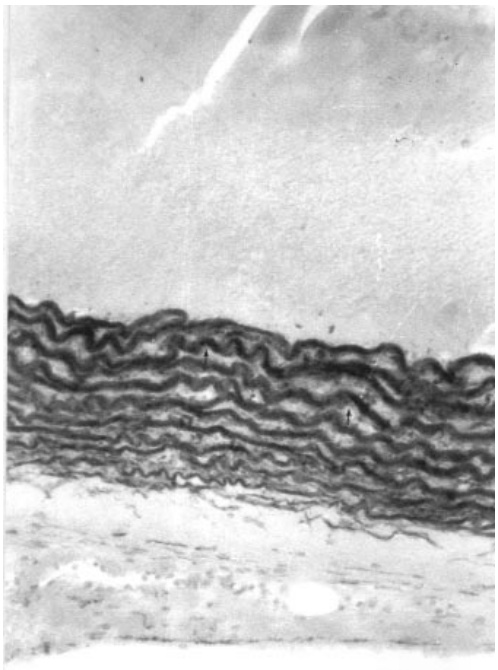

Figure 3. Control group. The media consists of concentrically arranged elastic laminae (arrows). Orcein X 66.

Discussion

NO is an important mediator for controling vascular resistance and is responsible for vasodilatation. The inhibition of NO production thus causes increased vascular resistance and increased arterial blood pressure (7). In our study the chronic inhibition of NO production by L-NAME caused higher mean arterial blood pressure. The increase of blood pressure in animals following chronic L-NAME administrations have been described in numerous studies (2,6,7). According to Babai et al. (2), oral administration of L-NAME in 40 (mg/kg)/day doses increased systolic blood pressure by 30% after the second week. Kristek et al. (8), using 50 mg/kg doses (half of the dose that we employed), reported significantly greater hypertension (about 187.2 mm Hg mean) in rats after 8 weeks of continual NO blockade with L-NAME.

In the present study we used two models for hypertension by suppressing NO production. Group II received 100 mg/kg L-NAME and group III received 500 mg/kg L-NAME. In both groups we found similar hypertension changes (Table), whereas different levels of NO inhibition were applied. Yoneyama et al. (9)

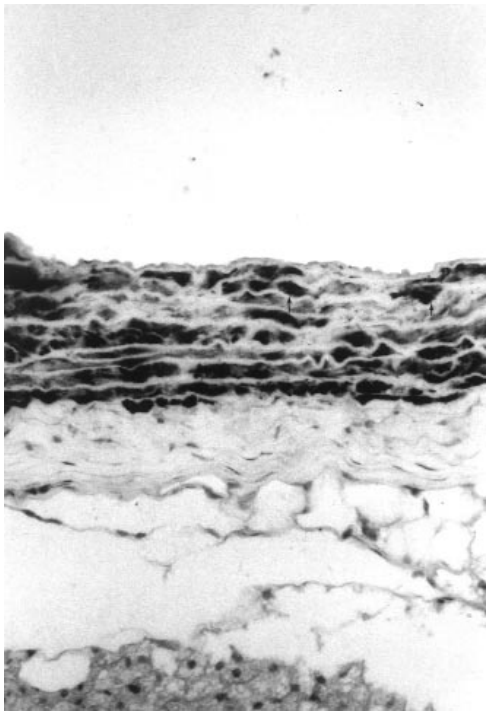

Figure 4. Control group. Smooth muscle cells stained with anti-α-SMA(+) can be seen between the elastic laminae (arrows). Immunostaining with anti-α-SMA X 66.

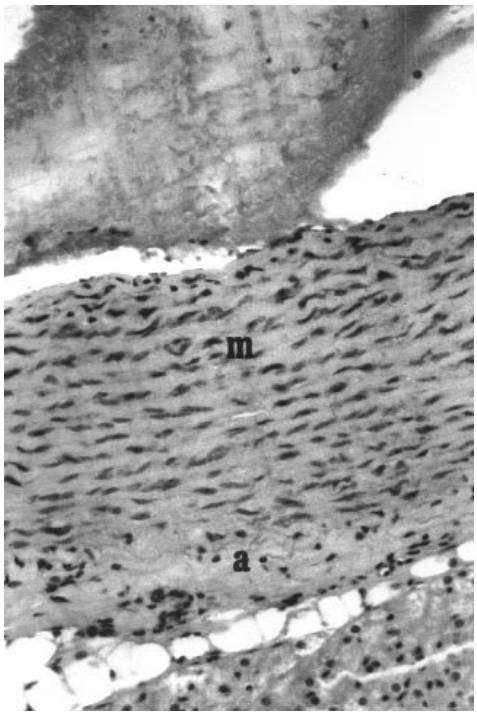

Figure 5. Group II. Notice the thick tunica media (m) where the adventitia is normal (a). H-E X66.

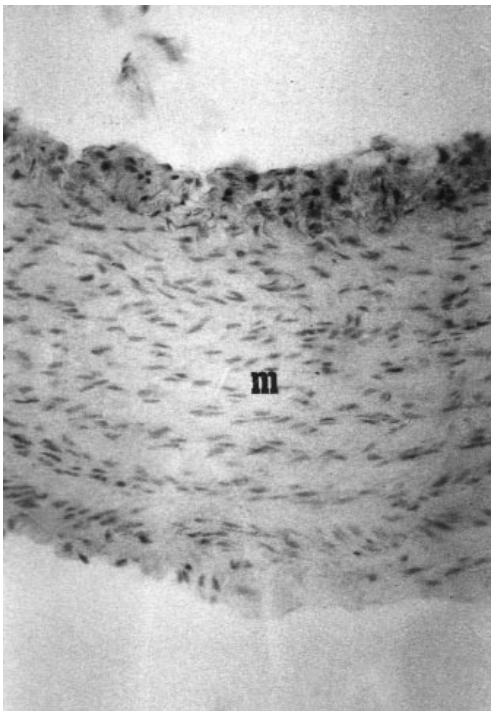

Figure 6. Group III. Medial thickening is more prominent (m). H-E X66.

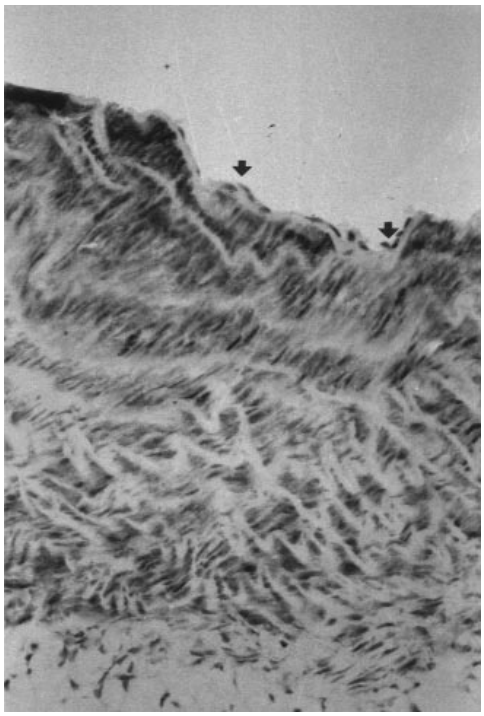

Figure 7. Group III. Notice the irregular luminal layers of the endothelial cell lining (arrows). Immunostaining with anti-α-SMAX66.

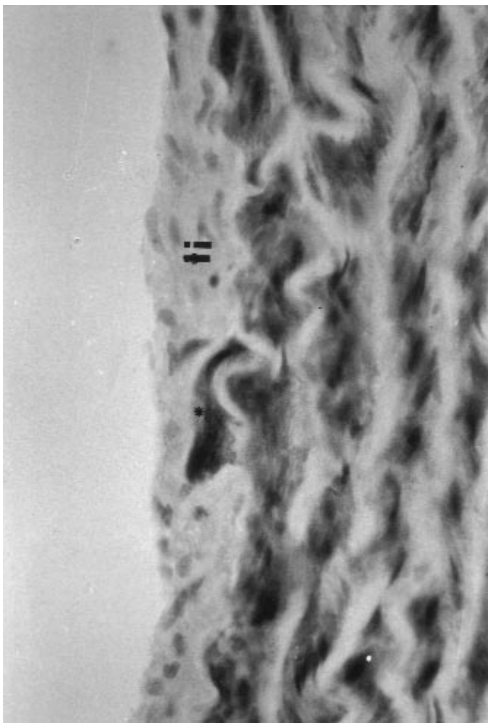

Figure 8. Group III. The thickening of the tunica intima is remarkable (ti). In this area anti-α-SMA (+) cells appear within the intima (*). Immunostaining with anti-α-SMA X 132.

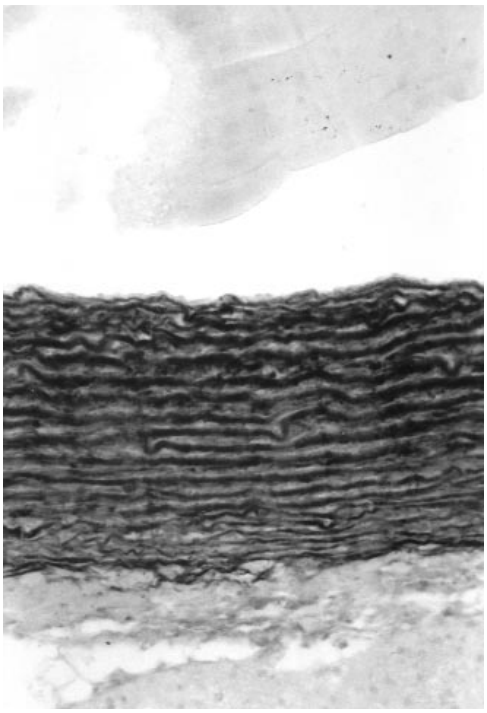

Figure 9. Group II. Increasing of the elastic laminae is marked. Orcein X 66.

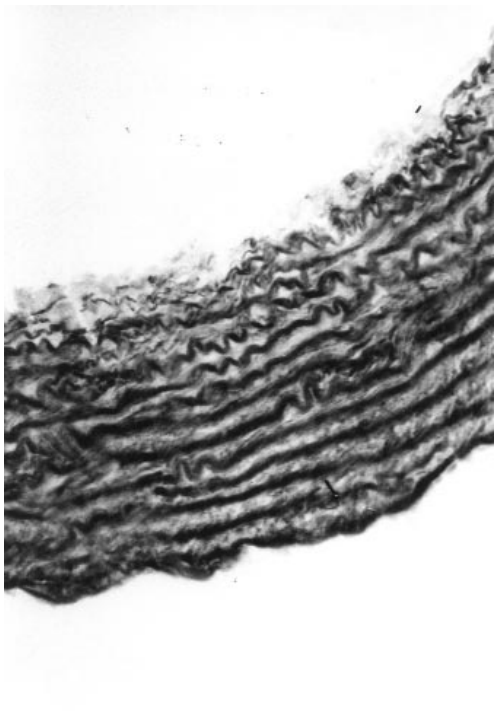

Figure 10. Group III. Increasing and thickening of the elastic laminae are more evident. Orcein X66.

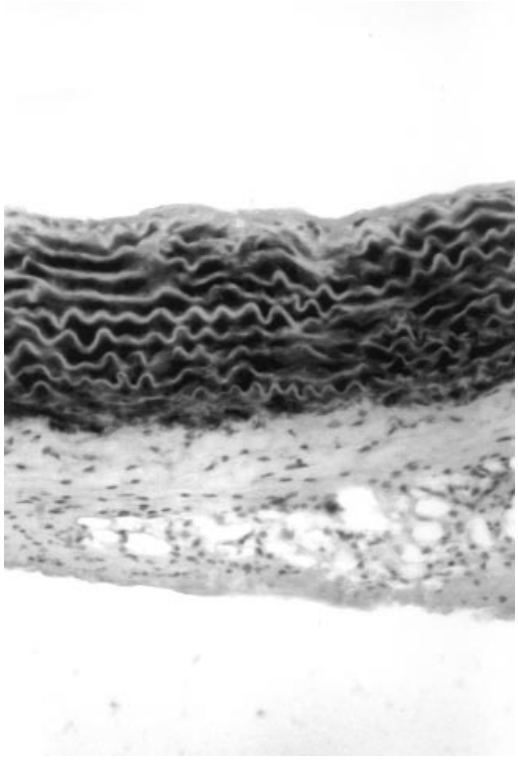

Figure 11. Group II. Intensity of anti-a-SMA labelling is increased. Immunostaining with anti-α-SMA X 66.

administered L-NAME to rats and found similar differences in the mean arterial blood pressure with moderate and severe NO inhibition. In contrast, Baylis et al. (5) reported dose dependency in the magnitude of the systemic hypertension with chronic NO blockade.

We also carried out a trial to separate the effect of decreased NO production on the vascular wall from that of hypertension induced by NO inhibition. As mentioned before, in the two models of L-NAME-induced hypertension the degree of hypertension was similar but the degrees of L-NAME doses were different. The degrees of vascular wall thickening were also different. Vascular wall thickening was more severe in group III than in group II. Yoneyama et al. (9) examined renal vascular wall thickening in L-NAME-induced hypertension. Although they found a correlation between the degree of vascular wall thickening and the dose of L-NAME, they were unable to find a correlation between the L-NAME dose and arterial hypertension, which is also supported by our study. Hypertension leads to medial hypertrophy resulting from increased numbers of smooth muscle cells and elastic fibers (10). However, we believe that the vascular wall thickening mainly occurred as a result of the

decreased NO synthesis, because in group III arterial hypertension was similar to that in group II, but arterial wall thickening was severe. It is possible that hypertension could have additive effects.

NO inhibits the proliferation of vascular smooth muscle cells (VSMCs), in part by a cGMP-dependent mechanism, although Na-K-ATPase activity may also be involved. NO also inhibits protein synthesis in VSMCs (2). It is possible that uncontrolled vascular medial smooth muscle cell proliferation led to the hyperplasia of the media in the thoracic aorta. Our results also demostrated that media thickneses were closely associated with the presence of dense fibrous tissue. In the elastic arteries, the media consists of sheets, or lamellae, of elastic material with intervening layers comprising smooth muscle cells, collagenous fibers and ground substance. The elastic lamellae are arranged as concentric fenestrated sheets (7). In our study the number of elastic lamellae increased according to the wall thickening. Bezie et al. (11) investigated the elastic lamellae in spontaneously hypertensive rats. They found no changes in the elastic densities between the control and spontaneously hypertensive rat groups.

In order to show the smooth muscle cell proliferation we performed anti-α-SMA immunostaining. α-SMC is the most abundant cellular protein of SMC and it has been widely used to identify the myofibroblast development in response to injury and in disease (12). We found an increased intensity of α-SMA labeling in both experimental groups.

In normal arteries the intima is composed of the lining endothelial cells with minimal underlying subendothelial connective tissue (7). There is evidence from experimental models of hypertension that the increased blood pressure stimulates the proliferation of cells in the tunica intima, possibly as a result of endothelial lesion caused by intravascular pressure (13). Guyton (14) reported that the subendothelium of the hypertensive thoracic aorta showed significantly increased volume measurements of mononuclear leukocytes and basement membrane-like material when compared with the control group. The inhibition of NO synthase exacerbates experimental intimal lesions. Both vascular smooth muscle cell proliferation and migration contribute to the lesion formation (3). Pereira et al. (13) detected an increased thickness of the intima of the small intramyocardial arteries following chronic NO inhibition. Rossi and Colombini- Netto (7) found prominent intima thickness in L-NAME-treated rat aortae. We detected focal thickening of the intima in some of the sections. In these areas connective tissue cells were seen but seldom anti-α-SMA positive cells.

In conclusion we detected that chronic NOS inhibition caused structural changes in the thoracic aorta wall in Wistar rats. These structural changes probably occurred as a result of the decreased NO production. However, hypertension could also have additive effects. The role of the NO in the vessel wall needs further investigation.

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