The anti-inflammatory and antioxidant effects of pravastatin and nebivolol in rat aorta

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ABSTRACT

Objective: The aim of this study was to investigate the effects of pravastatin and nebivolol in the atherosclerotic process including inflammation and oxidative stress in rat aorta.

Methods: This experimental randomized controlled study comprised of 35 Wistar albino rats. N^{ω}-nitro-L-arginine methyl ester (L-NAME) - induced vascular inflammation and arteriosclerosis were treated with both of the pharmacologic agents. All were divided into 5 equal groups: the control, group I: L-NAME -15 days, group II: L-NAME 30+ nebivolol, group III: L-NAME -30+ pravastatin, group IV: L-NAME - 30 days. Serum ceruloplasmin, uric acid, total antioxidant capacity (TAC), total cholesterol (T.Chol), low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG) were analyzed. Medial thickening and leukocyte infiltration status were examined histopathologically. The results were compared with control group and with each other using Kruskal-Wallis and Mann-Whitney U test.

Results: Pravastatin diminished the rise of ceruloplasmin, which was taken as an index of inflammation (p=0.002). Pravastatin and nebivolol decreased the L-NAME induced oxidative stress (p=0.001, 0.002, respectively). Nebivolol diminished the rise of LDL (p=0.04). Pravastatin lowered T.Chol, LDL and TG levels (p=0.001, 0.008, 0.040, respectively). HDL values were not changed significantly.

Conclusion: In conclusion, 15 days of statin therapy attenuated vascular inflammation and lowered the rised lipid levels (LDL, T.cholesterol and TG). Both the nebivolol and pravastatin exhibited antioxidant property. These documented beneficial effects of both of the drugs may improve the clinical outcomes of patients with hypertension or hyperlipidemia by additional studies. *(Anadolu Kardiyol Derg 2014; 14: 229-33)*

Key words: Antioxidants, atherosclerosis, ceruloplasmin, inflammation, statins

Introduction

Endothelial dysfunction is found to be the initializing factor in the pathological process of atherosclerosis, and the elevated inflammatory markers are shown to predict the future cardiovascular events (1). Due to inflammation, endothelium damages and the first visible lesion of the atherosclerosis, so called fatty streaks, occurs. According to the studies, it is reported that 50% of heart attack occurs in individuals with normal or even in low low density lipoprotein (LDL) values (2). Regarding the hypothesis in the pathogenesis of atherosclerosis, biomarkers other than LDL are categorized as inflammatory markers, oxidative stress markers, endothelial dysfunction markers etc. Therefore, the treatment process requires more than lipid lowering effect, but additionally antiinflammatory, and antioxidant effects. Interestingly lipid lowering has been found to have favorable effects on inflammatory process (1).

3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are the medical treatment of choice for hypercholesterolemia, because they are the pharmacological agents that have a highest LDL reduction power (3). The observational studies supported the concept of endothelial dysfunction, inflammation, and oxidative stress attenuation (4, 5). As a group, these drugs are different in their pleiotropic and lipid lowering effects (6-8).

Pravastatin is a drug belongs to the class of statins and has been shown to lower total and LDL cholesterol, triglycerides, and CRP levels, and has been shown to reduce the occurance of heart attacks and strokes caused by cardiovascular disease (6, 9). The antiinflammatory and antioxidant effects of pravastatin were determined in recent studies (10, 11). In an experimental study, pravastatin inhibited the H_2O_2-induced endothelial dysfunction (11).

Nebivolol, the β_1- receptor antagonist, is widely approved for the treatment of hypertension due to getting free nitro-oxide

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from dysfunctional endothelium (12). The pharmacologic properties of nebivolol include a nitric oxide-mediated vasodilatory effect and has been demonstrated to have an endothelium protection power with its antioxidant property (13-15). The determined antioxidant effects of nebivolol may contribute to a reduction in cardiovascular risk in hypertensive patients (16).

Inhibition of NO synthesis by N^{ω}-nitro-İ-arginine methyl ester (L-NAME) induces early inflammation and subsequent arteriosclerosis (medial thickening and perivascular fibrosis) in rats similar to humans (4). L-NAME inhibits the NO synthesis chronically in rats, thus inhibits its blocking effect on LDL oxidation and increases the oxidative stress (17, 18).

The present study is designed to investigate the effects of pravastatin and nebivolol on the L-NAME- induced vascular inflammation and arteriosclerosis in rat aorta in vivo with inflammatory and oxidative stress markers and histological examinations. We hypothesized that both of the pharmacological agents have pleiotropic effects which were not much investigated though they were widely used by patients.

Methods

Study protocol and animals
The present study protocol was reviewed and approved by the Ethics Committee on animal experimentation. In this experimental randomized controlled study, 35 female (to standardize the gender) Wistar-Albino rats (180-200 g) were obtained from an established colony from Animal Research Institution of Ege University Hospital.

Study design
The rats were housed in cages in a quiet room at a controlled temperature (21±2°C) in a 12-12 h light-dark cycle during 30 days. L-NAME (Pfeizer, USA), Nebivolol (Vasoxen, Ulagaylar, Turkey) and Pravastatin Sodium (Provachol, Pfeizer, USA) were the used pharmacological agents. All drugs were prepared freshly every study day and were dissolved in distilled water to give orally (gavage) with an orogastric tube.

Rats (n=35) were randomly divided into 5 groups:

Control group (n=7): Received standard food and drinking water.

Group 1 (n=7) (L-15): Received standard food plus L-NAME (30 mg/kg per day) in the drinking water for 15 days.

Group 2 (n=7) (L30+N15): Received standard food plus L-NAME (30 mg/kg per day) for the first 15 days, and L-NAME (30 mg/kg per day) + Nebivolol (20 mg/kg per day) for the last 15 days.

Group 3 (n=7) (L30+P15): Received standard food plus L-NAME (30 mg/kg per day) for the first 15 days, and L-NAME (30 mg/kg per day) + Pravastatin Sodium (30 mg/kg per day) for the last 15 days.

Group 4 (n=7) (L-30): Received standard food plus L-NAME (30 mg/kg per day) for 30 days.

After 30 days of feeding, serum levels of total cholesterol (T.Chol), triglycerides (TG), high density cholesterol (HDL), uric acid, ceruloplasmin and total antioxidant capacity (TAC) were measured. Then the rats were anesthetized and euthanized for morphometric and immunohistological analyses.

Biochemical analysis
Serum T.Chol, TG, HDL, uric acid assays were performed on Abbott Aeroset (Abbott, Wiesbaden, Germany) according to the manufacturer's specifications by using proprietary reagents. Concentration of LDL was calculated by using the Friedewald formula $[(LDL)]=(T.Chol)-(HDL)-[(TG)/5]$ where all concentrations are given in mg/dL (19).

Ceruloplasmin: measured by immunoturbidimetric assay method on Abbott Aeroset and expressed as mg/dL.

Total Antioxidant Capacity (TAC): Serum TAC was measured using a colorimetric measurement method developed by Erel (20) on Abbott Aeroset. The results are expressed as mmol Trolox eq./L.

Histopathological examination
The rats were euthanized and abdominal aorta segments were fixed in 10% neutral buffered formalin solution. The sections were embedded in paraffin and sections of 6μ were moved onto slides. The slides were stained with Gomory's Trichrome, Hematoxylin Eosin and examined and photographed by Olympus BX51 TF photomicroscope. Medial thickening and leukocyte infiltration status were examined.

Statistical analysis
The distribution of the variables were determined using Shapiro-Wilks normality test. Kruskal Wallis test followed by posthoc Mann-Whitney U test was used to compare the quantitative data. All data were presented as mean±SD. A level of $p<0.05$ was considered statistically significant. Analyses were performed using SPSS for Windows, version 11.0 (SPSS Inc., Chicago, IL, USA).

Results

Effects of statins on inflammatory and proliferative changes
Figure 1 shows Gomory's Trichrom stained aortic segments from the experimental groups. In the control rats, no evidence of inflammation was observed. In L-30 group, more leukocyte infiltration was observed than L-15 group. In groups treated with antioxidants, leukocyte infiltration was lesser. Medial thickening (the wall-to-lumen ratio) was widest in L-30 group (p=0.002) (Fig. 2A). Pravastatin treatment ameliorated these changes (Fig. 1, 2) (Table 1).

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Table 1. Serum levels of the analytes and tunica media thickness of rat abdominal aorta

Analytes	Control n=7	L-15 n=7	L-30+N-15 n=7	L-30+P-15 n=7	L-30 n=7	*P
T.Chol, mg/dL	60±5	68±2	54±8	45±5**†¥	60±1	0.001
HDL, mg/dL	22±4	23±4	23±5	23±3	22±4	0.990
LDL, mg/dL	25±5	31±1	15±6¥	12±6**¥	28±1	<0.001
TG, mg/dL	62±9	67±2	75±3	57±2†	53±2	0.159
Uric acid, mg/dL	1.65±0.26	1.87±0.16	1.98±0.36	2.10±0.83	1.60±0.85	0.164
Ceruloplasmin, mg/dL	2.0±0.8	4.7±0.9**¥	3.5±1.0**	3.1±0.5**†	3.8±0.8**†	<0.001
TAC, mmol Trolox eq./L	0.82±0.16	0.59±0.14**	0.74±0.01†¥	0.88±0.04†¥	0.64±0.06**	<0.001
Medial Thickness, µm	297±21	341±21**¥	475±80**†	414±16**†	483±103**†	<0.001

T.Chol-total cholesterol; TG - triglyceride; TAC - total antioxidant capacity, L-30-L-NAME -30 days, L-30+N-15- L-NAME 30+ nebivolol, L-30+P-15: L-NAME -30+ pravastatin
**p<0.05 vs. control, †p<0.05 vs. L-15 group, ¥p<0.05 vs. L-30 group.
Data are means±SD. Significant differences are marked in bold font. p<0.05 is considered as significant. Abbreviations: L-15: L-NAME -15 days
*Kruskal Wallis test was used for multigroup comparison, Mann-Whitney U test was used for bi-group comparison
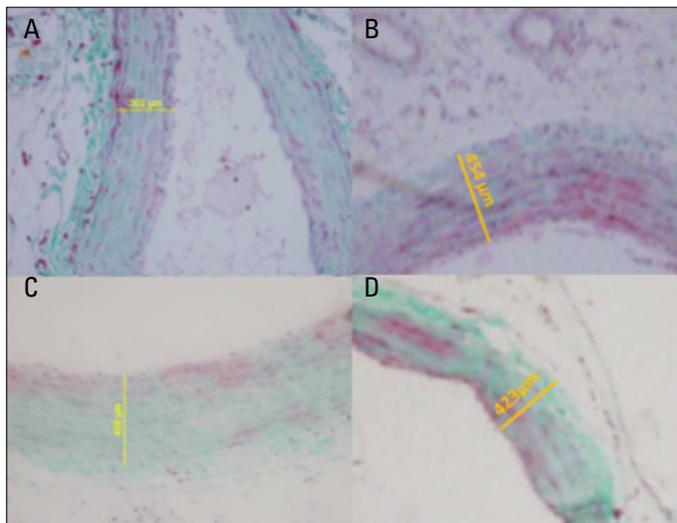
Figure 1. Histopathological photomicrographs of abdominal aorta sections stained with Gomory's Trichrom; control (1A), L-30 (1B), L-30+N-15 (1C), L-30+P15 (1D)

Serum lipids
L-NAME modified the lipid levels of the rats and pravastatin lowered their T.Chol, LDL and TG levels (Fig. 2B-D). HDL values were not changed significantly. Nebivolol diminished the rise of LDL (p=0.04) (Table 1).

Uric acid
No significant change was determined (Fig. 2E).

TAC
Pravastatin and nebivolol decreased the L-NAME induced oxidative stress (Table 1, Fig.2F).

Ceruloplasmin
Pravastatin diminished the rise of ceruloplasmin, which was taken as an index of inflammation (p=0.002) (Table 1) (Fig. 2G). There was also a tendency to lower ceruloplasmin levels by nebivolol; however, it did not reach statistical significance.

Discussion

We demonstrated in the current study that histological examinations revealed a thickening of intima and media, and leukocytes infiltration with L-NAME administration in rat aorta.

Pravastatin treatment suppressed the L-NAME- induced high lipid levels and increased the L-NAME reduced antioxidant capacity. Pravastatin also decreased the L-NAME increased ceruloplasmin levels. Nebivolol showed an antioxidant effect besides its LDL lowering effect in this rat model.

Although ceruloplasmin and uric acid have antioxidant effects, these may work as prooxidants in the atherosclerotic process (21). Ceruloplasmin, the acute-phase protein, has been shown to oxidize LDL and cause vascular injury by generating free radicals (22). However, the role of ceruloplasmin in lipoprotein oxidation in the atherosclerotic process is uncertain (23). In a study, serum ceruloplasmin levels were found as high in diabetic acute myocardial infarction patients whose antioxidant levels were found as lower (24). Similarly, in this study, low levels of antioxidant capacity is accompanied with high serum ceruloplasmin levels. In a study, authors examined the role of ceruloplasmin in patients with cardiomyopathy and determined the high ceruloplasmin values correlated with the extent of heart failure (25). With pravastatin treatment, L-NAME induced high ceruloplasmin levels were decreased, and we can say that ceruloplasmin appears to be correlated with inflammation in this experiment.

Uric acid is an independent risk factor for cardiovascular disease especially in high risk patients, and hyperuricemia is associated with endothelial dysfunction (26). In a study by Maxwell et al. (27), an association with cardiovascular disease and high serum uric acid levels was examined and they suggested that it might be due to a consequence of an impairment of vascular nitric oxide activity. In contrary to that study, no significant change was observed in serum uric acid concentration during this experiment. Similar to our study, Moutzouri et al. (28) found no effect on uric acid levels with statin treatment in patients with coronary artery disease for which they considered as a risk factor with elevated levels. In a study by Cuenca et al. (29), serum high uric acid levels

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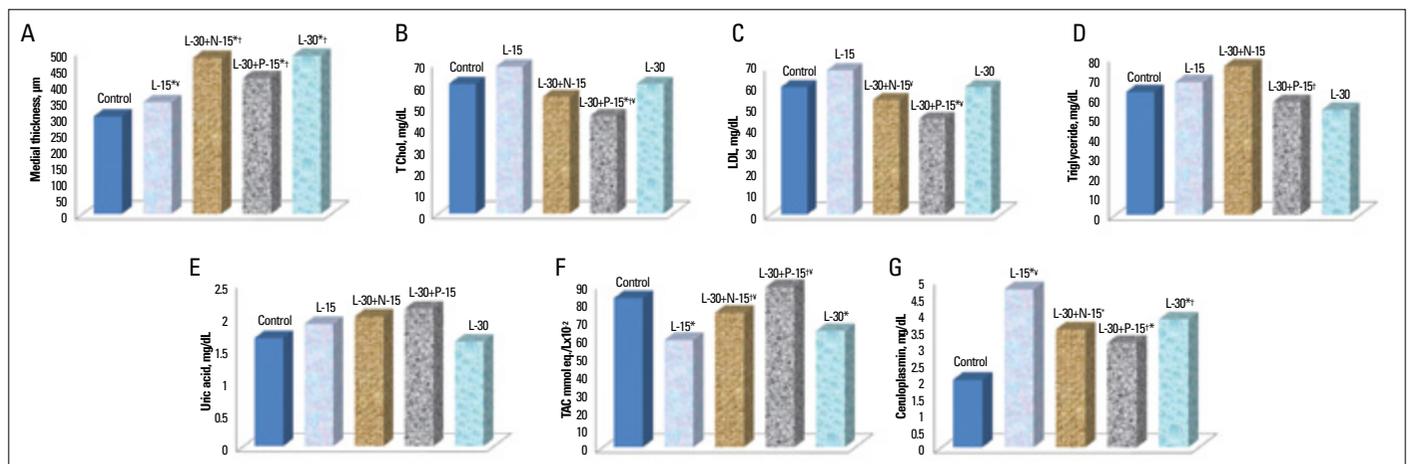
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Figure 2. The effects of Pravastatin and Nebivolol on inflammatory and proliferative changes; medial thickness (2A), T Chol (2B), LDL (2C), triglyceride (2D), uric acid (2E), total antioxidant capacity (2F), ceruloplasmin (2G).
*p<0.05 vs. control
¥p<0.05 vs. L-NAME 30 days
†p<0.05 vs. L-NAME 15 days

were decreased with high dose statins in patients with ischemic heart disease irrespective of its lipid lowering effects.

Statins effectively reduce the cardiovascular events of patients with coronary heart disease and hypercholesterolemia. Their antiatherogenic potential is not exclusively dependent on their lipid-lowering properties, but exerts pleiotropic effects on vascular and cardiac cells independent of cholesterol synthesis. Moreover, statins reduce vascular smooth muscle cell proliferation and reactive oxygen species production (30-32). Büyükhatipoğlu et al. (33) examined the antioxidant effects of statins in patients with coronary artery disease. The atorvastatin treatment caused an increase in total antioxidant capacity and they concluded that the rise in ceruloplasmin levels was independent of its antioxidant property.

In the current study, treatment with pravastatin inhibited markedly the L-NAME- induced high total and LDL cholesterol levels although statins are demonstrated to have no lipid lowering effects in rats even at high doses (34). Triglyceride levels were also decreased with pravastatin treatment, but no effect was observed in HDL levels in this study.

Nebivolol attenuates the endothelial dysfunction and the vascular oxidative stress (35). The nitric oxide-mediated vasodilatory activity differs this drug from other beta blockers, hence this action can result in interference with lipid metabolism (36). The hypertension exhibits hyperlipidemia, therefore hypertensive therapy requires lipid lowering therapy in patients. In a study (36), nebivolol was compared with atenolol, and authors considered nebivolol to be more advantageous with its lipid lowering and antiinflammatory effects in addition to its antihypertensive effects. In an experimental study, nebivolol was shown to prevent oxidative stress in rats with ischemia-induced cerebral injury (37). The present study indicates that treatment with nebivolol increases the total antioxidant capacity, and loweres the L-NAME induced high LDL levels.

In the current study, histopathologically the leukocyte infiltration reflected the presence of inflammation. A marked infiltration was observed in the perivascular area in the L-30 group. Furthermore, high ceruloplasmin levels accompanied with an increased oxidative stress. That increase in mononuclear leukocytes was reduced by treatments with both of the drugs.

Study limitations

As a limitation of the current study, the results are based on small number of rats, and the experiment was made with only female rats to avoid gender differences, therefore, the results might not be evaluated according to male rats.

Conclusion

Fifteen days of statin therapy attenuated vascular inflammation and lowered the rised LDL T.cholesterol and TG concentration. Both the nebivolol and pravastatin exhibited antioxidant property. These documented beneficial effects of both of the drugs may improve the clinical outcomes of patients with hypertension or hyperlipidemia by additional studies.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

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