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The impact of rosuvastatin on inflammatory markers in L-NAME induced hypertensive rats

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Abstract

Background and objective: Many clinical trials have revealed that HMG-CoA reductase inhibitors (statins) have anti-inflammatory effects through their pleiotropic activities there by decreasing the risk of cardiovascular disease (CVD). This study intended to evaluate the effect of rosuvastatin on the level of inflammatory markers (hsCRP, IL-6, sCD40L, Lp-PLA2, and cystatin C) in normotensive and hypertensive rats.

Methods: Twenty-four male Wister rats were divided into two groups of twelve. Group 1 consisted of normotensive rats, while Group 2 served as the hypertensive model. Each group was further subdivided into two groups. Subgroup A served as the control group which received the only placebo and subgroup B was the treatment arm which received rosuvastatin 10mg/kg daily for 4 weeks.

Results: Rosuvastatin did not significantly affect blood pressure and heart rate in both normotensive and hypertensive rats. The level of inflammatory markers (hsCRP, IL-6, and Lp-PLA2) significantly increased in hypertensive rats, while the level of both sCD40L and cystatin C did not change. Rosuvastatin lowered the level of IL-6, sCD40L, Lp-PLA2, and cystatin C significantly in hypertensive model rats. However, the level of hsCRP was non-significantly reduced by rosuvastatin. In normotensive rats treated with rosuvastatin, the level of cystatin C was significantly reduced.

Conclusion: Rosuvastatin significantly decreased the level of IL-6, Lp-PLA2, sCD40L, and cystatin C in hypertensive rats while in normotensive rats, rosuvastatin treatment produced only a reduction of cystatin C. Our results suggest an anti-inflammatory effect of rosuvastatin in hypertention through reduction of inflammatory markers.

Keywords: Rosuvastatin, Hypertension, Inflammatory markers.

Introduction

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also known colloquially as statins, are the primary therapy option for hypercholesterolemia. The main effect of statins is to lower low-density lipoprotein cholesterol (LDL-C).[1]

Many studies confirm that statins significantly lower the rate of occurrence of cardiovascular (CV) events in high-risk individuals with raised plasma cholesterol concentrations with or without affirmed cardiovascular disease (CVD).[2,3,4] The beneficial effect of statins in these trials was mostly due to their ability to decrease LDL cholesterol levels by inhibiting hepatic LDL cholesterol synthesis. However, recently the decrease in cardiovascular events by statins is postulated to be due to pleiotropic effects that are not directly connected to their lipid-lowering properties.[5] These 'pleiotropic effects' include reduction of the vascular inflammatory processes, improvement of endothelial function, anti-oxidant and antithrombotic actions, and plaque stabilization.[6]

Both hypertension and dyslipidemia are the major threat to cardiovascular disease.[7]

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Although there are multiple causes forhypertension, the literature suggests vascular inflammation and atherosclerosis have a crucial role in its pathogenesis and progression.[8] Measuring the level of high sensitivity CRP (hs-CRP) and IL-6 with other circulating inflammatory biomarkers are suggested to be useful for detecting the risk of developing hypertension.[9] A landmark randomized-control clinical trial, the JUPITER trial, concluded that rosuvastatin can reduce first major CV events and death in patients with raised hs-CRP, even with LDL cholesterol levels lower than the current threshold indications for treatment, suggesting that statins may have an additional beneficial role in the reduction of CV events through their anti-inflammatory effects.[10]

Further evidence demonstrates that HMG-CoA reductase inhibitors, the preferred drug option in the management of hyperlipidemia, can also reduce blood pressure in hypertensive patients, thereby reducing the risk of cardiovascular disease.[11, 12] This effect is likely related to their anti-inflammatory properties by reducing vascular inflammation, which involves improvement of endothelial function, and reduction of oxidative stress and inflammation in patients with hypertension, but with average levels for cholesterol.[13]

Inflammatory biomarkers, particularly C-reactive protein (CRP) and IL-6 provide clinicians with vital information regarding inflammatory processes that may underlie and predict future cardiovascular events and coronary artery disease.[14] C-reactive protein, an acute-phase inflammatory protein synthesized by liver cells, is well established as a pro inflammatory marker with increased serum levels serving as an effective independent predictor of cardiovascular disease.[15] CD40 and its immunomodulating ligand (CD40L) take part in the progress of coronary atherosclerosis and provoking acute coronary syndrome and they are involved in the atherothrombotic process that may connect inflammation, atherosclerosis, andthrombosis.[16] Lp-LPA2 is an enzyme secreted by inflammatory cells such as macrophages and is associated with atherosclerotic plaque expansion and rupture.[17] Both atherosclerosis and hypertension are associated with kidney diseases, therefore early detection before disease progression is important. Recently, serum Cystatin C level has been shown to provide compelling evidence for early detection of renal problems.[18, 19]

Many clinical trials in humans investigated whether serum concentrations of inflammatory markers, such as high sensitivity-CRP (hs-CRP), cytokines (IL-6), soluble CD40 ligand, cellular adhesion molecules (ICAM-1), and sA2 phospholipases (Lp-PLA2) can predict the risk of cardiovascular events and coronary artery disease.[20, 21, 22]

If statins have been demonstrated to reduce CV events in patients with high CRP levels, and CRP as an inflammatory marker has been associated with the development of atherosclerosis which is implicated in CV events, then it is reasonable to presume that statins may reduce the incidence of CV events by reducing inflammation through pleiotropic effects. In this study, the impact of rosuvastatin on the level of these inflammatory markers in hypertensive experimental rats has been evaluated.

Methods

In this study, male albino rats with a bodyweight ranging from 200–300 grams were used. Rats were kept in the animal house of Hawler Medical University, College of Medicine. The room temperature was maintained at 20-25° C. A 12 hour light/dark cycle was set. Rodent food rich in nutrients and tap water was used as feeding.

Twenty-four male Wister rats were divided into two groups of twelve. All rat groups were fed a standard diet for one week before starting the experiment as an acclimatization period for adaptation.

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Group 1 consisted of normally-fed, normotensive model rats, while Group 2 served as the hypertensive model. Hypertension was induced in Group 2 subjects by giving Nω-nitro-L-arginine (L-NAME) 40mg/kg/day.[23] Each group was further subdivided into subgroup A and subgroup B. Subgroup A served as the control group which received the only placebo which consisted of distilled water administration, and subgroup B was the treatment arm which received rosuvastatin 10mg/kg daily orally by gastric gavage for 4 weeks.[24]

Blood pressure (systolic, diastolic, and mean blood pressure), heart rate, and body weight were recorded at the beginning of the study and were re measured at the end of treatment for all groups. Blood pressure was measured by using the tail-cuff method (CODA non-invasive blood pressure monitor/Kent Scientific Corporation, USA). The rats were put in a Plexiglas restraining cage for at least 20 minutes before each blood pressure recording. The occlusion cuff was placed at the base of the tail and the VPR sensor cuff (specially designed differential pressure transducers measure the blood volume in the tail) was placed adjacent to the occlusion cuff.[25]

Four parameters were recorded during the blood pressure measuring, which are: systolic, diastolic, mean blood pressure, and heart rate. For each rat at least 5 readings of blood pressure were taken.[26] On the last day of treatment for each group, Xylazine 10 mg/kg and 125 mg/kg Ketamine injections were used for anesthetization of the rats.[27] Blood was withdrawn through a cardiac puncture. The collected blood was centrifuged then the serum was used for serologic testing, including the level of hsCRP, IL-6, Lp-LPA2, sCD40L, and cystatin C by using ELISA rat kits (Enzyme-Linked Immunosorbent Assay).

Statistical Analysis

The analysis of Data were performed using The Statistical Package for Social Sciences (SPSS version 26). The data were expressed as mean ± SE. Data analysis was made using one-way analysis of variable (ANOVA). The comparison between groups were done using Tukey test. A *P*-value of 0.05 or less was considered statistically significant.

Results

Changes were observed in blood pressure and heart rate, however, statistically not significant in normotensive Group 1B rats after treatment with rosuvastatin for 4 weeks. In Group 2A, the L-NAME induced hypertensive rat group, the systolic, diastolic and mean blood pressure were significantly (P <0.05) increased in comparison to Group 1A, the normotensive control group, demonstrating the success of hypertension induction, though no significant change in heart rate was appreciated. However, no statistically significant difference in blood pressure and heart rate was appreciated between group 2A and group 2B, despite treatment with rosuvastatin (Table 1).

The level of the inflammatory marker, hsCRP, significantly (P = 0.02) increased in Group 2A hypertensive control rats compared to Group 1A normotensive control rats, suggesting increased inflammation in the hypertensive model. However, no significant change in hsCRP was noted in both Group 2B hypertensive and Group 1B normotensive treatment arm (Figure 1).

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Table1 Effect of Rosuvastatin (10mg/kg) on blood pressure and heart rate in normotensive and L-NAME induced hypertensive rats

Parameters	Group 1A- Normotensive Control	Group 1B – Normotensive treatment arm (Rosuvastatin treated)	Group 2A - Hypertensive Control	Group 2B - Hypertensive treatment arm (Rosuvastatin-treated)
Systolic blood pressure (mmHg)	124.0±4.95 a	121.1±12.41 a	158.5±3.66 b	161.8±4.31 b
Diastolic blood pressure (mmHg)	91.6±5.17 a	96.3±17.14 a	120.1±5.60 b	121.8±6.07 b
Mean blood pressure (mmHg)	102.0±5.05 a	98.3±15.47 a	131.5±4.87 b	136.3±4.55 b
Heart rate Beat/Minute	356.8±12.71 a	381.3±50.59 a	330.8±4.81 a	338.8±8.26 a

-The different letters in the table indicate the significancy at *P* >0.05

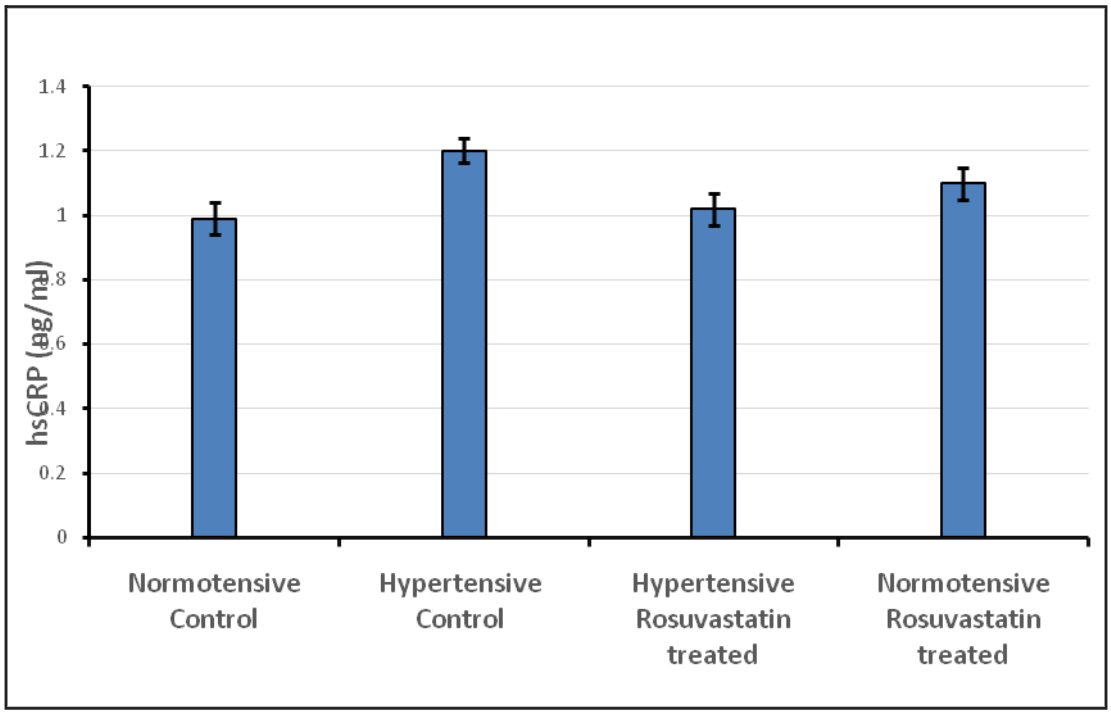

Figure 1 Effect of Rosuvastatin(10mg/kg) on hsCRP in L-NAME induced hypertensive rats

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A significant increase in the level of IL-6 (P = 0.02) and Lp-PLA2 (P = 0.025) was observed in Group 2A hypertensive control rats compared to the Group 1A normotensive control. The level of both IL-6 (P = 0.01) and Lp-PLA2 (P = 0.004) significantly decreased in Group 2B hypertensive rats treated with rosuvastatin. However, there was no statistically significant change in group 1B normotensive rats treated with rosuvastatin (Figure 2, 3).

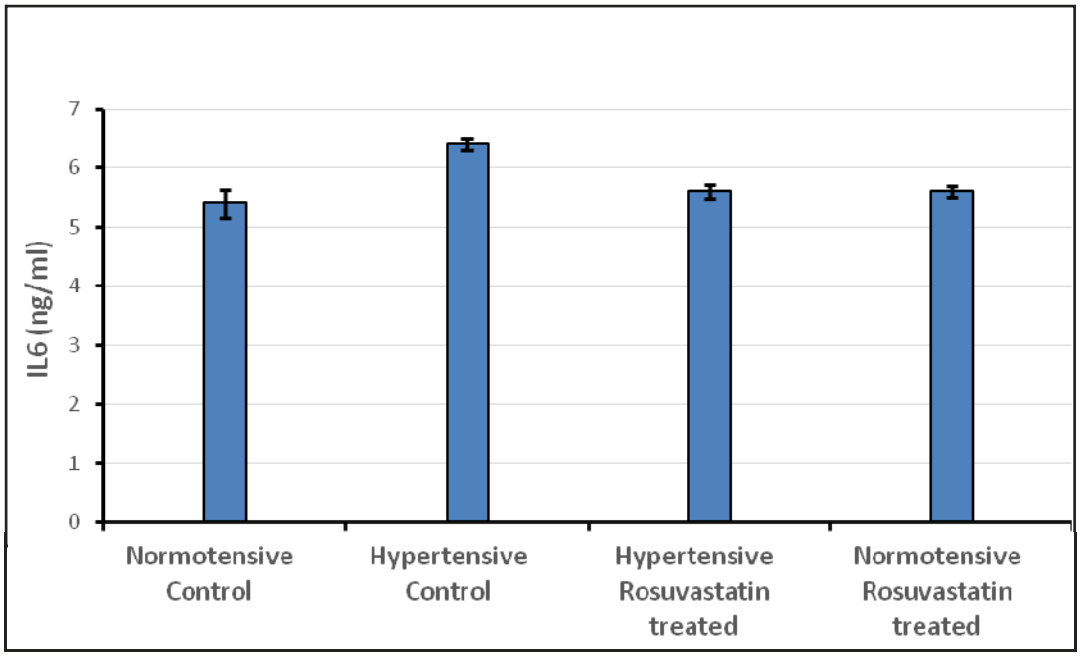

Figure 2 Effect of Rosuvastatin(10mg/kg) on IL6 in L-NAME induced hypertensive rats

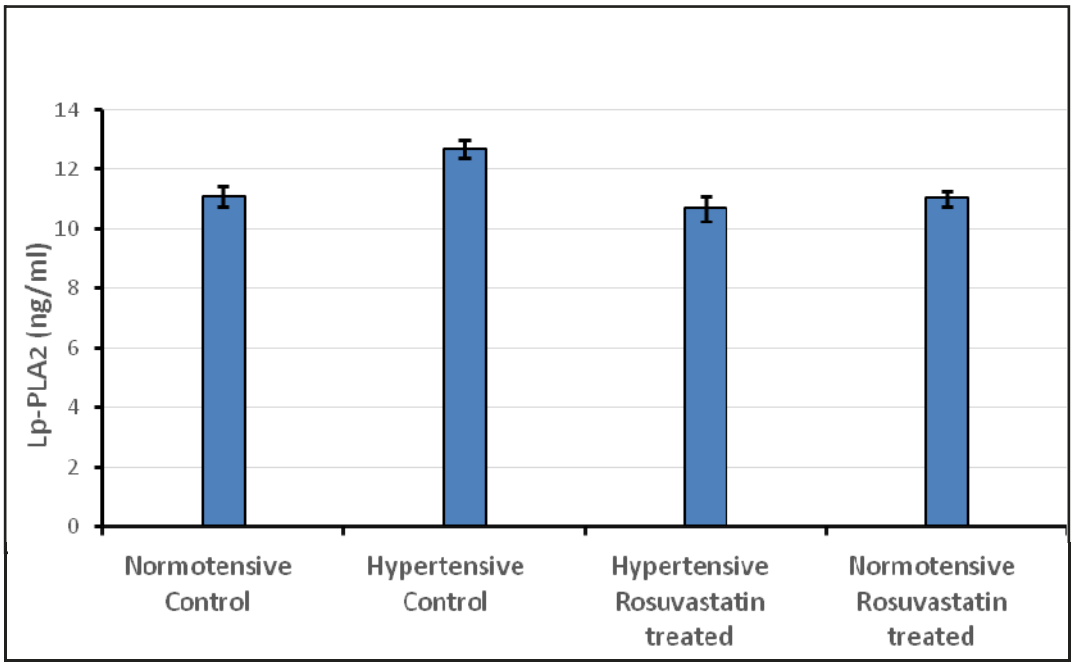

Figure 3 Effect of Rosuvastatin (10mg/kg) on Lp-PLA2in L-NAME induced hypertensive rats

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No changes were appreciated in the level of sCD40L and Cystatin C in Group 2A hypertensive control rats compared to the Group 1A normotensive control, but their levels were significantly (*P* = 0.001) reduced in Group 2 B hypertensive rats treated with rosuvastatin. Also, the level of cystatin C significantly (*P* = 0.015) decreased in Group 1B normotensive rats treated with rosuvastatin while there was no change in sCD40L (Figure 4, 5).

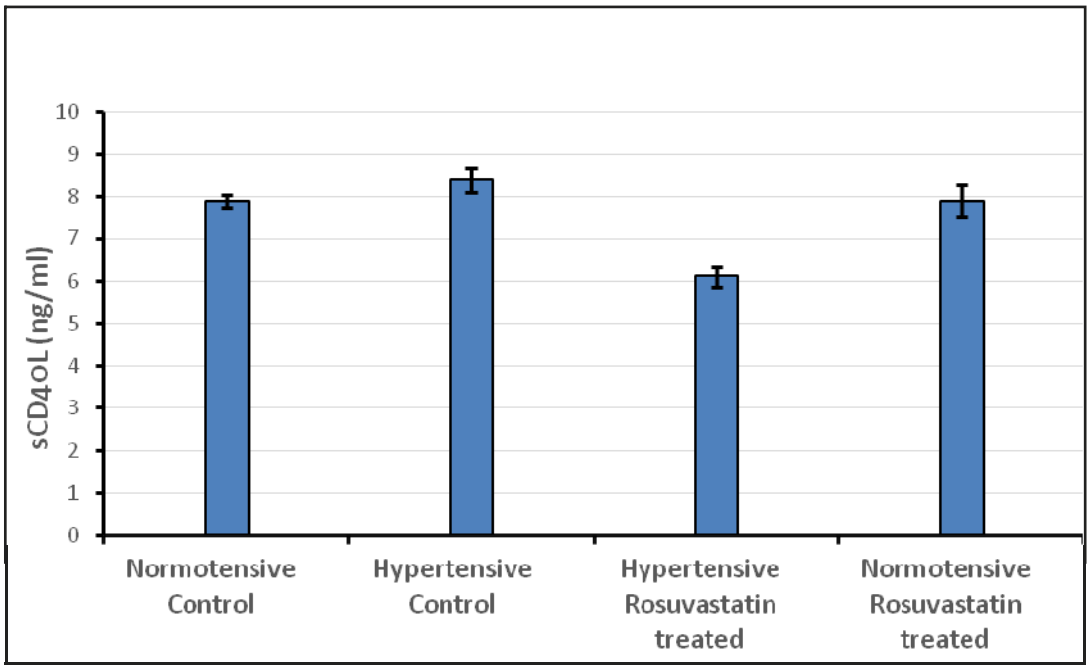

Figure 4 Effect of Rosuvastatin (10mg/kg) on CD40Lin L-NAME induced hypertensive rats

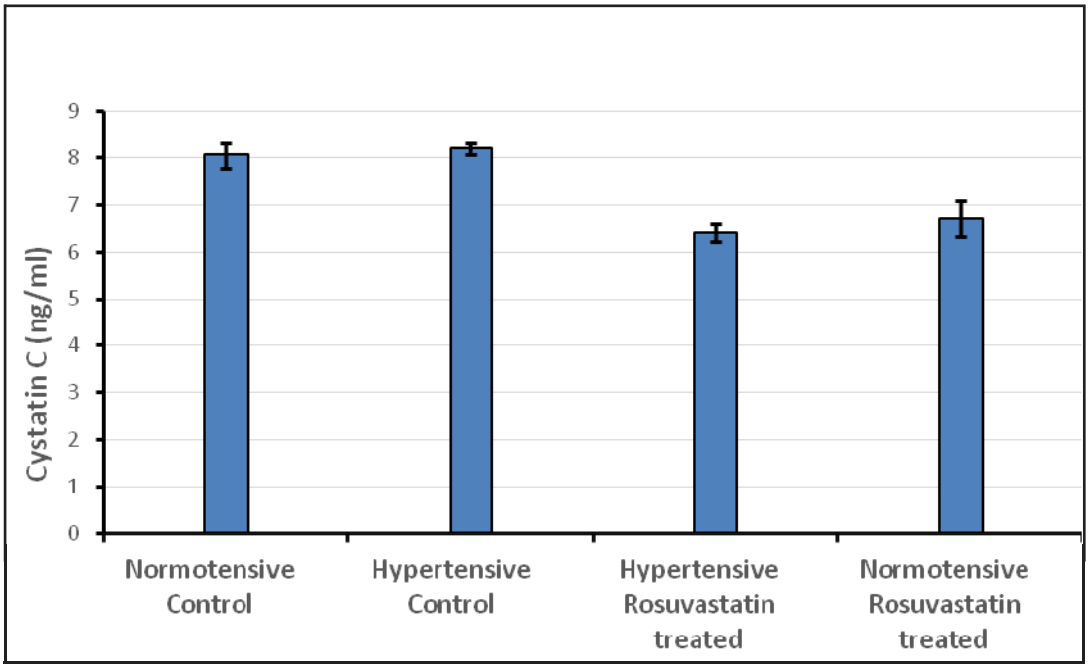

Figure 5 Effect of Rosuvastatin (10mg/kg) on Cystatin C in L-NAME induced hypertensive rats

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Discussion

In the present study, the systolic, diastolic, and mean blood pressure in the L-NAME induced hypertensive rat group significantly (P <0.05) increased compared to the normotensive control group. L-NAME is an esterified form of Nω-nitro-L-arginine methyl ester. L-NAME-induced hypertension is a prevalent model of experimentally induced hypertension.[28] L-arginine analog, a non-selective inhibitor, causes hypertension by suppressing the activity of nitric oxide synthase (NOS) both in vitro and in vivo.[29]

In this study rosuvastatin, an HMG-CoA reductase inhibitor did not produce any significant changes in systolic, diastolic, mean blood pressure, or heart rate in either hypertensive or normotensive model rat groups. There are contradictory pieces of evidence about the influence of HMG-CoA reductase inhibitors (statins) on blood pressure. A randomized, double-blinded, placebo-controlled trial has assessed the impact of simvastatin and pravastatin on blood pressure and revealed a great reduction in both systolic and diastolic blood pressure.[30] A meta-analysis of placebo-randomized control trials demonstrated the effect of statins on reducing both systolic and diastolic blood pressure.[31] The mechanism of lowering blood pressure has contributed to an improvement in the endothelial function and reducing oxidative stress in hypertensive patients, by increasing the endothelial production of nitric oxide (NO) and up-regulation of endothelial NO synthase expression.[32] In agreement with our study; however, Banach et al. (2013) showed no significant effect of statins in reducing blood pressure.[33] Some other studies also reported that statins did not reduce blood pressure significantly in normotensive hypercholesterolemic patients, or in hypertensive patients controlled with anti-hypertensive medications.[34,35] You et al. (2017) in a meta -analysis also concluded that both atorvastatin and simvastatin could reduce blood pressure but not rosuvastatin.[36] These differing conclusions about the effect of statins on blood pressure may be due to a variety of causes e.g. restrictions in the methods of the study including small sample size, and insufficient time of statin treatment.[37] Some of these researches have indicated that statins could lower blood pressure by improving endothelial dysfunction, increasing nitric oxide production, and decreasing oxidative stress, which is regarded as one of the mechanisms that are involved in the pathogenesis of hypertension.[38] However, in the present study, L-NAME induced hypertension in the rats by inhibiting endothelial nitric oxide synthase activity, the inability of rosuvastatin to lower the blood pressure in the hypertensive rats might be due to the possibility that L-NAME has already inhibited nitric oxide synthase enzyme.

The hsCRP blood level measures low-grade systemic inflammation, without the presence of an obvious systemic inflammatory or immunologic disorder. hsCRP is the most commonly evaluated biomarker in cardiovascular disease (CVD) risk prediction.[39]

In this study, the level of hsCRP and IL-6 were significantly (P <0.05) raised in the hypertensive control group compared to the normotensive control group. These findings are supported by a study performed on 385 hypertensive and 196 normotensive individuals, which has shown that higher than standard levels of IL-6 and C-reactive protein (CRP) were seen in hypertensives compared with normotensives.[40] Also, a recent meta-analysis indicated that higher levels of IL-6 and CRP increase the risk of hypertension.[41] Additionally, an increase in the level of hsCRP is associated with endothelial dysfunction, and it is well-known that endothelial dysfunction has a crucial role in the development of hypertension by reducing the production of NO.[42, 43] This supports the conclusion that inflammation is likely implicated in the

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development of hypertension.

Statins have an established anti-inflammatory effect that slows the progression of disease.[44] In the PRINCE trial, oral uses of pravastatin for 24 weeks significantly lowered serum CRP levels in subjects with and without CVD.[45] In this study, however, there was no statistically significant decrease in hsCRP following rosuvastatin treatment in both hypertensive and normotensive rats, but there was a significant reduction in the level IL-6 and LpPLA2. LpPLA2 enzyme is specific for arterial inflammation. It is also regarded as a promising biomarker for assessing the development of CVD because it indicates the presence of an unstable atherosclerotic plaque.[46]

Our findings also showed no significant changes in the levels of sCD40L and Cystatin C in the hypertensive control rats compared to the normotensive control group. Alternatively, Huang, et al. (2018) detected that high circulating levels of sCD40L were found in patients with white coat hypertension.[47] While in another study there was no rise in the level of sCD40L in newly diagnosed hypertensive patients.[48] Despite the fact that the CD40-CD40L system, as well as its soluble mediator sCD40L, is engaged in the key inflammatory and thrombotic pathways of atherosclerotic cardiovascular illnesses,[49] there is still controversy about its role as a risk factor for hypertension and CVD. Furthermore, many studies demonstrated that serum cystatin C is more useful than serum creatinine for evaluating renal function. A higher level of cystatin C in the blood is linked to an increased risk of cardiovascular disease.[50, 51] Also, a high circulating concentration of cystatin C has been detected in hypertensive patients, which may reveal mild kidney dysfunction without clinically obvious kidney disease.[52,53] However, in the current study, no change was detected in the level of cystatin C in hypertensive control rats which may indicate that the kidneys have not yet been affected by hypertension, which progressively affects major organs including the kidneys.[54]

Additionally, in the present study, although the level of sCD40L and cystatin C did not significantly change in hypertensive control rats compared to normotensive controls, there was a significant reduction noted in the hypertensive model treated with rosuvastatin. This result suggests an anti-inflammatory effect of rosuvastatin through reduction of inflammatory markers, independent of its lipid-lowering effect, this indicates that although rosuvastatin could not lower blood pressure, it could reduce vascular inflammation—which has a pivotal role in pathogenesis of hypertension.

Conclusion

Rosuvastatin had no significant effects on blood pressure and heart rate in both normotensive and hypertensive rats. The levels of inflammatory markers like hsCRP, IL-6, and Lp-PLA2 were significantly increased in hypertension-induced rats. Rosuvastatin significantly decreased the level of inflammatory markers, IL-6, Lp-PLA2, sCD40L, and Cystatin C in hypertensive rats. However, in normotensive rats, rosuvastatin treatment resulted only in the reduction of cystatin C.

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Competing interests

The authors declare that they have no competing interests.

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