Effect of statin on vascular wall thickness in kidney disease model

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Abstract. Fibrosis is a major histopathologic feature of kidney disease especially chronic renal failure. Fibrosis can occur in the glomerulus, tubules, interstitial tissue and blood vessels. Fibrosis in blood vessels is usually characterized by thickening and loss of elasticity of muscular arteries walls. This study focus to assess the effect of statin on the wall thickness of blood vessels in kidneys tissue with the animal model of kidney disease. A total of 18 male swiss was used in these study. All the animal divided into 4 groups: sham group (S, n=4), subtotal nephrectomy (SN, n=4), simvastatin group 10 mg/kgBB (SIM10, n=5), and simvastatin group 20 mg/kgBB (SIM20, n=5). The kidneys tissue is stained with picrosirius red staining. Wall thickness on vascular kidneys is measured at the end of the study by Image J software. After 2 weeks of study, we found that the fraction area of wall thickness in SO group is significantly lower compared with the SN group (8.35±1.47 vs 26.86±6.39, p<0.000). While in the SIM group, treatment with statin also led to decrease of wall thickness significantly compared with the SN group (SIM10: 17.32±0.58, p<0.003; SIM20: 13.45±2.27, p<0.000). This finding suggests that administration of statins in kidney disease may improve the wall thickness on kidneys tissue.

1. Introduction
CKD is a progressive disorder affecting almost 14% of the general population. Patients with CKD have higher rates of hospitalization, greater mortality, and shorter life expectancy. [1] Fibrosis is a major histopathologic feature of chronic renal failure. Fibrosis can occur in the glomerulus, tubules, interstitial tissue and blood vessels. This pathophysiologic process is associated with changes in the structure of renal vasculature due to abnormal accumulation of extracellular matrix (particularly collagen types I, III, and IV) [2].

Fibrosis in blood vessels is usually characterized by thickening and loss of elasticity of muscular arteries walls. This thickening and loss of elasticity occur in two distinct sites, the intimal and medial layers of the vasculatures. Intimal calcification is associated with atherosclerotic plaques and medial calcification is characterized by vascular stiffening and arteriosclerosis.[3] Endothelial dysfunction is a key step in the initiation and maintenance of atherosclerosis. Multiple factors, including circulating inflammatory cytokines, TNF-α (tumor necrosis factor-α), reactive oxygen species, and oxidized LDL
(low-density lipoprotein), can activate endothelial cells, leading to impaired vascular relaxation, increased leukocyte adhesion, increased endothelial permeability and generation of a pro-thrombotic state [4].

Previous studies in chronic renal disease with different etiologies have shown that renoprotective effects of statin to prevent interstitial fibrosis, tubular injury, and glomerulosclerosis. [5] However, it is not yet known how the statins effect on fibrosis in renal blood vessels. This study aims to determine the effect of statin on the condition of renal blood vessels especially wall thickness using animal models.

2. Methods

2.1. Ethical statement
This study was approved by The Medical and Health Research Ethics Committee Faculty of Medicine, Universitas Gadjah Mada.

2.2. Experimental Protocol
A total of 18 male swiss was used in these study. At 3-5 month of age, the mice were randomly allocated to 5/6 subtotal nephrectomy (SN), sham operation (SO), 5/6 subtotal nephrectomy with 10 mg/kgBB simvastatin (SIM10), and 20 mg/kgBB simvastatin (SIM20). The animals kept in a temperature-controlled room at 22±2°C, humidity at 55±5%, with a 12-h light/dark cycle and allowed free access to water and a standard mouse diet.

For 5/6 nephrectomy, we made an incision on a flank region of the skin. The right kidney was removed after ligation of the renal blood vessels and the ureter. The upper and lower poles of the left kidney were resected, leaving an intact kidney segment. After 2 weeks of treatment, all the animals were terminated by intraperitoneal injection of sodium pentobarbital 0.1 ml/10g body weight.

The rest of the kidney was embedded in paraffin for histopathologic examination. Paraffin-embedded renal tissue was dewaxed using standard sequential techniques and 4 µm-thick sections were stained with picrosirius red staining. Using a 400X magnification light microscope, 5 renal vascular field sightings were assessed. Analysis using software Image J. The perimeter wall and lumen of the blood vessel was assessed to find the wall area and perimeter center. The wall thickness of blood vessels is obtained by dividing the value of the wall area and perimeter center. The result of this calculation is a fraction.

2.3. Statistical Analyses
We used the SPSS 21.0 software for data processing. The results were expressed as the mean±standard deviation (SD). The results were analyzed by one-way ANOVA followed by Tukey test and p<0.05 was considered significant.

3. Results
Based on analysis using Image J software, we found data about vessel area, lumen area, perimeter wall and perimeter lumen of the blood vessel as shown in Table 1.

| Parameter | SO (n=4) | SN (n=4) | SIM10 (n=5) | SIM20 (n=5) |
|-----------|----------|----------|-------------|-------------|
| Vessel area | 118.88±84.3 | 301.68±206.7 | 324.03±271.69 | 165.89±140.23 |
| Lumen area | 50.01±43.84 | 150.96±119.15 | 149.82±165.53 | 65.93±59.92 |
| Perimeter wall | 50.26±27.6 | 71.07±31.08 | 72.03±34.81 | 50.05±24.43 |
| Perimeter lumen | 33.01±21.51 | 59.51±28.51 | 50.66±29.66 | 35.58±19.13 |

SO, sham-operated animal; SN, 5/6 subtotal nephrectomy; SIM10, 5/6 subtotal nephrectomy with 10 mg/kgBB simvastatin; SIM20, 5/6 subtotal nephrectomy with 20 mg/kgBB simvastatin
After further analysis, we found that there were significant differences in wall thickness between SO group and SN group at the end of the study (p<0.000). The wall thickness on SO group was 8.35±1.47 and on SN group was 26.86±6.39. There were significant differences between the SIM group and SN group (SIM10: 17.32±0.58, p<0.003; SIM20: 13.45±2.27, p<0.000). But there is no significant difference between the simvastatin groups (p<0.29).

**Figure 1.** Comparison of the perimeter wall and perimeter lumen of blood vessels

**Figure 2.** Comparison of the wall thickness of blood vessels between group
Figure 3. Comparison of blood vessel in treatment group (A= 5/6 subtotal nephrectomy (SN); B=sham-operated animal (SO); C=5/6 subtotal nephrectomy with 10 mg/kgBB simvastatin (SIM10), D= 5/6 subtotal nephrectomy with 20 mg/kgBB simvastatin

4. Discussion

The endothelium is the largest organ in the body strategically located between the wall of blood vessels and the bloodstream. [6] The endothelium plays a critical role in maintaining normal vascular homeostasis including on renal vascular. [7] Hypertension, inflammation, diabetes-associated factors, and uremic toxins are some risk factors for endothelial dysfunction in chronic kidney disease. [6] Uremia in CKD patient with can accelerates atherogenesis by modulates the smooth muscle cells (SMCs). This cell plays a key role in the progression of atherosclerosis [8,9].

In the last few decades, the effects of statins have been widely studied, especially in kidney disease. Simvastatin treatment reported improving renal endothelial function via a reduction in oxidative stress in a model of obesity and hypertension. [10] Pitavastatin reduces inflammation within atherosclerotic lesions in CRD mice. [11] Rosuvastatin improves endothelial function in db/db mice by the role of angiotensin II type 1 receptors and oxidative stress. [12] These effects because of the endothelial Krüppellike factor 4 (Klf4). Endothelial Klf4 is renoprotective and mediates statin-induced protection by regulating the expression of cell adhesion molecules and the concomitant recruitment of inflammatory cells [13].

Another study also found that statin therapy significantly improves dyslipidemia and brachial artery endothelial function in patients with nephrotic syndrome. Improvement in brachial artery endothelial function may be in part related to a non-lipid effect of statins. [14] Treatment with statins also reduced endothelial microparticles (EMPs) levels in CKD patients. This effect could be understood by mechanisms that prevent the release of EMPs from the damaged endothelium or by a direct effect on circulating EMPs [15].
5. Conclusion
In conclusion, we have shown that treatment with statin led to a decrease in wall thickness significantly compared with the SN group. This finding suggests that administration of statins in kidney disease may improve the wall thickness on kidneys tissue.

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