RESEARCH ARTICLE

Determination of Endothelin-1 after Treatment with Rosuvastatin in Induced Hyperlipidemic Rats

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ABSTRACT

Aim of the study: The objective of this study is to investigate the correlation between rosuvastatin and endothelin-1 on endothelium and their relation to oxidative stress in hyperlipidemic male rats.

Materials and methods: Thirty male wister rats are used in this research, their age about 1 month Thegrouping which are: group 1/ rats receiving normal diet pellets, group 2/ Dyslipidemic rat of high-fat diet group 3/ hyperlipidemic rats with Rosuvastain dose (0.86 mg) Group 4/ hyperlipidemic rats with Rosuvastain dose (1.29 mg), group 5/ hyperlipidemic rats with Rosuvastain dose (1.72 mg). Experiment duration was 10 weeks from induction of dyslipidemia until the end of treatment. Endothelin-1 levels were measured by ELISA technique.

Results: Rosuvastatin decreases the endothelin-1 level in all treated groups (G3,G4,G5) while increases the level of endothelin-1 in control positive (dyslipidemia G2) so the results of endothelin levels are significant increases ($p < 0.05$) between control negative group 1 (63.08 ± 9.172) and control positive dyslipidemia group 2 (89.54 ± 39.652) and no significant differences between rosuvastatin groups (G3,G4,G5).

Conclusion: The rosuvatatin improved the endothelium of blood vessel and reduced the oxidative stress that generated due to the hyperlipidemia.

Keywords: Endothelial dysfunction, Endothelin-1, Dyslipidemia. Rosuvastatin.

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Conflict of interest: None

INTRODUCTION

Dyslipidemia is heterogeneous group of disorders that are characterized by high lipid levels inside bloodstream. Also the term hyperlipidemia denoted to the high concentrations of lipids (triglycerides, cholesterol, or both) in the blood vessels. This disorder of lipoprotein metabolism, including lipoprotein overproduction or deficiency.[1] Lipids like cholesterol, cholesterol esters, phospholipids, and triglycerides. also, the hyperlipidemia is described as pro inflammatory state in which increased oxidative stress and endothelial dysfunction is seen.[2]

The dysfunction of endothelial cells in the vasculature is profoundly implicated in the pathogenesis of cardiovascular disorders.[3] Mounting evidence has shown that endothelial cell dysfunction is characterized by disrupted vascular tone and redox balance, and increased inflammatory reactions within the blood vessel wall.[4] The Endothelin-1 (ET-1), the main member of the endothelin peptide family, is a 21-amino-acid peptide with a hydrophobic C terminus and 2 cysteine bridges at the N terminus. Endothelins also include ET-35 as well as vasoactive intestinal contractor (4) the murine analog of human ET-2.[6] The mature ET-1 peptide is synthesized by almost every cell type, with high expression in vascular endothelial and smooth muscle cells, airway epithelial and smooth muscle cells, macrophages, fibroblasts, cardiac myocytes, mesangial cells, podocytes, and brain neurons.[7,8] ETA receptors primarily promote vasoconstriction, inflammation, and cell proliferation, the ETB receptor can be considered its endogenous, physiological antagonist because its activation by ET-3 inhibits the a for mentioned ET-1- mediated effects.[7] ET-1 and its receptors are widely expressed in tissue in macrophages, vascular smooth muscle cells, fibroblasts, and circulating white blood cells.[9] Endothelial cell and vascular smooth muscle cell expression of ET-1 is increased in patients with ischemic heart disease.[10,11] The aim of the current study is that to assess the effect of rosuvastatin in endothelial functions.

MATERIALS AND METHODS

Study Design

Thirty male wistar rats were used in this research, their age about 1 month the experimental model divided into 5 groups depend on the differences of quality of diet contents and treatment doses: group 1/the rats receiving normal diet

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pellets (normal diet contents- 50% (w/w) wheat, 42.15% (w/w) corn, 5%(w/w) soyabean, 2.5% (w/w) vegetable protein, 0.25% (w/w) antioxidants, and 0.1% (w/w) multivitamins) from week 1-14. group 2/ the hyperlipidemic rats get high fat diet (pellet with sun flower oil & Cholesterol & stearic acid) and oral gavaging by sunflower oil daily. group 3/ the hyperlipidemic rats + Rosuvastain dose (0.86 mg), group 4/ the hyperlipidemic rats + Rosuvastain dose (1.29 mg), group 5/ the hyperlipidemic rats + Rosuvastain dose (1.72 mg) (using oral gavage, 10–20 mg/kg bodyweight per day) during the same period.

Before starting the experiment, the rat located for 10 days under controlled room temperature 19–22 degree centigrade and light cycle of 12 hours light: 12 hours dark.

The blood sampling was done until the required range of cholesterol achieved above 50 mg/dL to get the induction step. Endothelin-1 was assessed using enzyme immunoassay kit, lipoproteins were analyzed in serum. Markers of oxidative stress,MDA,[12] GSH,[13] SOD[14] were assessed calorimetrically.

The abdominal aorta was dissected from animal rats and they were fixed in 10% neutral buffered formalin, processed by standard procedure of paraffin embedding, and serial sections were cut (5 µm). The sections were stained with hematoxylin and eosin dye and viewed under light microscope with magnification of 100× for histological changes.[15]

Statistical Analysis

The results are expressed as MEAN ± S.D. Differences between groups were assessed by ANOVA using the SPSS software package for Windows. Post hoc testing was performed for intergroup comparisons using the least significance difference test with Pb.05 considered as significant.

RESULTS

Endothelial Function Biomarkers

According to results of present study, that serum endothelin-1 levels were significantly increased ($p < 0.05$) between of normal control group 1 (63.08 ± 9.172) and control positive of hyperlipidemia group 2 (89.54 ± 39.652) comparing to rosuvastatin treated groups (G3,G4,G5). No significant differences between rosuvastatin groups (G3,G4,G5).

Serum malondialdehyde (MDA) results of present study, revealed that the significant increased among 0 groups in dyslipideamia group 2 comparing to normal control group 1 (p value < 0.05),the control positive group showed the highest mean (1.26 ± 0.238) nmol/mg) while the control negative group showed the lowest mean (0.71 ± 0.09 nmol/mg).

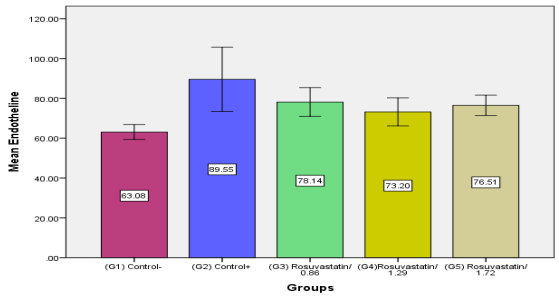

Figure 2: The mean of endothelin-1

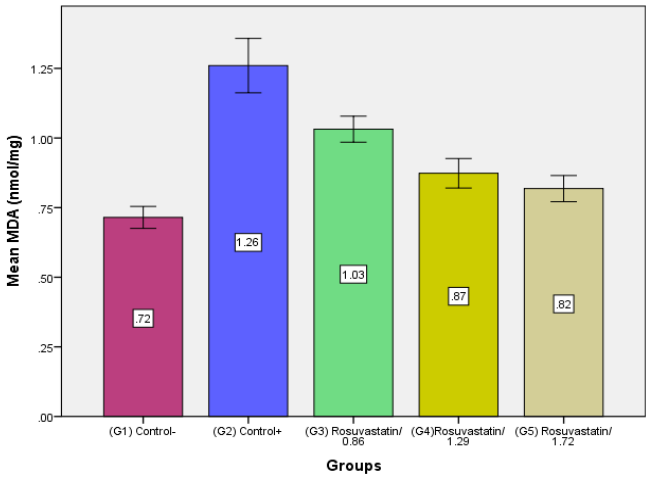

Figure 3: The mean of MDA

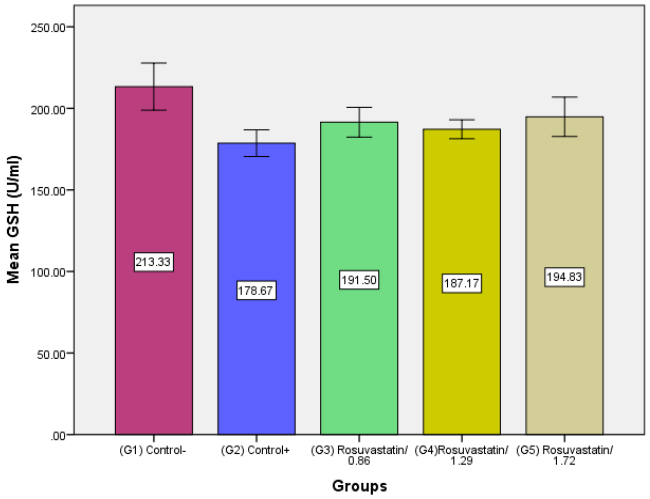

Figure 4: The mean of GSH

Table 1: The mean ± SD of biochemical markers in the current study

Grouping	*Endothelin-1*	*MDA*	*GSH*	*SOD*
(G1) control -	63.08 ± 9.172b	0.71 ± 0.096c	213.33 ± 35.387a	1.63 ± 0.175a
(G2) control +	89.54 ± 39.652a	1.26 ± 0.238a	178.66 ± 20.136b	1.78 ± 0.331a
(G3) Rosuvastatin 0.86 mg	78.14 ± 17.733ab	1.03 ± 0.114b	191.5 ± 22.394ab	1.83 ± 0.332a
(G4) Rosuvastatin 1.29 mg	73.19 ± 17.209ab	0.87 ± 0.129bc	187.1 ± 14.274ab	1.83 ± 0.150a
(G5) Rosuvastatin 1.27 mg	76.51 ± 12.509ab	0.81 ± 0.115c	194.8 ± 29.552ab	1.53 ± 0.216a
LSD	26.17	0.17	30.25	0.30

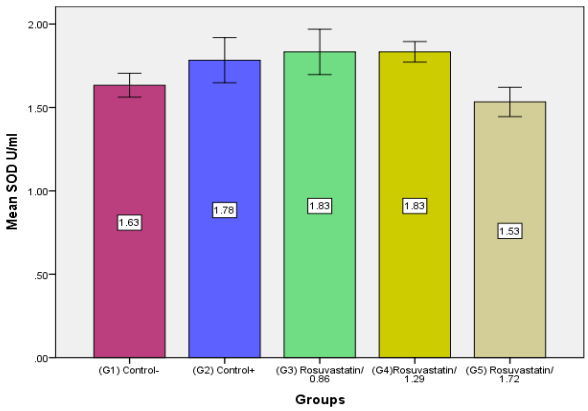

Figure 5: The mean of SOD

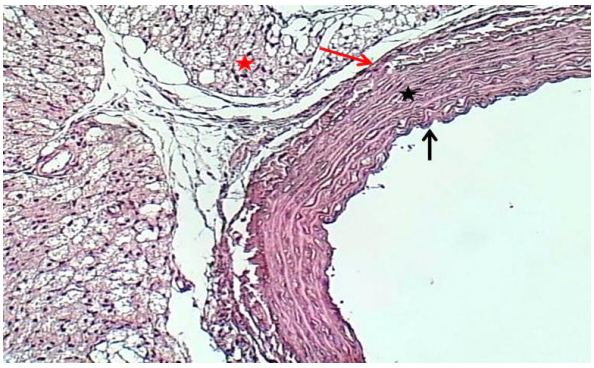

Figure 6: Section of cross section of aorta (Control group 1) shows: tunica intima (Black arrow), tunica media (Black asterisk), tunica adventitia (Red arrow) & perivascular brown adipose tissue (Red asterisk) H&E stain.100x

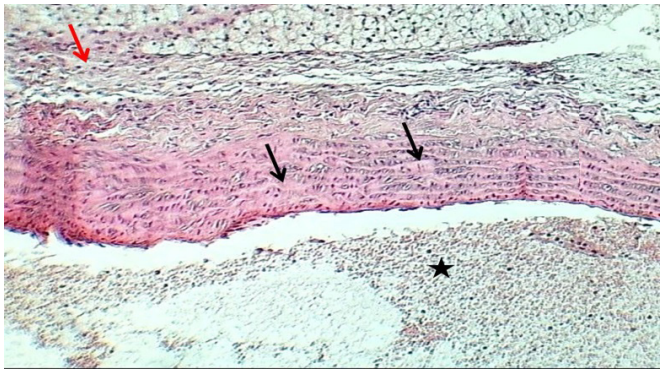

Figure 7: Longitudinal section of aorta (control positive group 2) shows: luminal thrombus formation (Asterisk), degeneration of elastic lamella and muscle fibers (Black arrows) and mild periarteritis (Red arrow) H&E stain.100x.

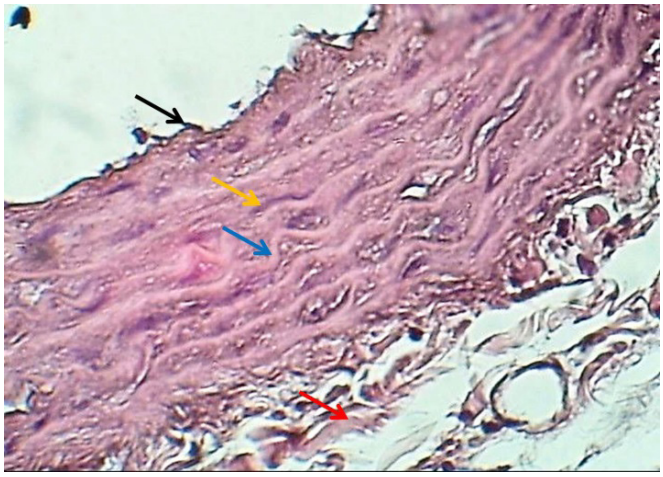

Figure 9: Longitudinal section of aorta (Group 5- RSV treatment with 1.72 mg) shows normal endothelial cell (Black arrow), normal smooth muscle fibers (yellow arrows), elastic lamella (Blue arrows) & adventia (Red arrow). H&E stain. 400x.

No significant differences between control negative group 1 and RSV group5,there was significant decreases in MDA levels in RSV group G3,G4 comparing to dyslipidaemia group 2.

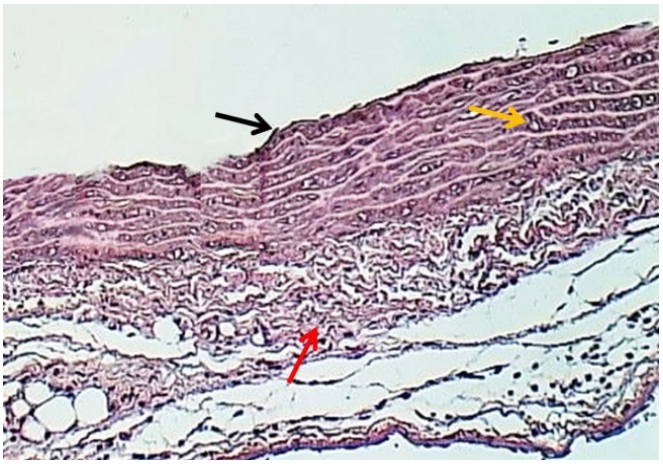

Figure 8: Longitudinal section of aorta (group 4- RSV treatment with 1.29 mg) show normal endothelial lining of tunica intima (Black arrow), mild degenerative changes of smooth muscle fibers (Yellow arrow) & normal adventia (Red arrow).H&E stain.100x.

Serum glutathionethe (GSH) results revealed that the differences in glutathione levels among groups were significant decreased ($p<0.05$) in dyslipidaemia group 2 comparing to normal control group 1.The control negative group showed the highest mean (213.33 ± 35.387 U/mL) while the control group positive showed the lowest mean (178.66 ± 20.136 U/mL). Slight significant increases of GSH levels in RSV groups (3,4,5) comparing to normal control group 1.

Regarding the SOD levels of present study, the results revealed that no significant differences in superoxide dismutase enzyme among groups

There will be slight significant increased in G2,G3,G4,G5 comparing to normal control group 1.

Normal histological structure of (control negative group 1) the aorta has displayed in Figure 6.

DISCUSSION

In the current study, endothelin-1 levels increase in dyslipidemia group compare to group 1 since oxidative stress follow the event of hyperlipidemia which decreases the bioavailable nitric oxide and leads to potentiate ET-1 signaling. This evidence suggests the link between ET-1 and

oxidative stress.[16] This agreed with recent data which indicated that hypercholesterolaemia increases endothelin-1 levels and endothelin receptor activity.[17] Black *et al*. suggested Oxidative stress could be associated with alterations in the ET-1 signaling pathways, and conversely, ET-1-induced vasoconstriction may be dependent on the production of superoxide anion.[18]

Elevated level of malonedialdehyde was observed in all hyperlipidemia groups then it was decreased in rosuvastatin group (G3,G4,G5) and the lowest level notified in last group of rosuvastatin G5 compare to control negative group G1. This agreed with previous study that found a significant reduction of MDA concentrations in patients treated with statins both when considered as a single class and when categorized based on their physicochemical properties, that is, hydrophobic statins (simvastatin and atorvastatin) that might be dispersed at low concentrations throughout human tissues, and hydrophilic statins (pravastatin, rosuvastatin and fluvastatin) that primarily target the liver and are found in the circulation.[19]

The GSH levels elevated with different doses of rosuvastatin groups (G3,G4,G5) however it decreased in dyslipidaemia (group 2) so the rosuvastatin dose 1.27 mg was the best for improving the GSH levels.

Rahman, *et.al* 2006 suggest that glutathione (GSH) was the most important non enzymatic antioxidant and can be used as an indicator of oxidative stress.[20] This agreed with data's that suggest the statins prevented GSH reduction or GSSG production So that rosuvastatin was the best drug for experimental model since it lessen both inflammation and oxidative stress parameters.

The Serum superoxide dismutase's (SOD) level slightly elevated in dyslipidemia group 2 and all different doses of rosuvastatin groups (G3,4,5) compared to control negative group.

Rosuvastatin is able to restore the antioxidant defense by improving SOD1 expression thereby providing protection against oxidative stress, which is contributor to post ischemic injury in the heart.[21] Rosuvastatin enhances the expression of glutathione synthase, glutathione peroxidase, glutathione reductase and glutamylcysteine synthetase, the rate limiting enzyme of glutathione synthesis.

In conclusion, the rosuvatatin was one of lipid lowering agents with anti-inflammatory and antioxidants properties that can improve the endothelium of blood vessel and reduced the oxidative stress that generated due to the hyperlipidemia.

REFERENCES

1. Araújo-Vilar D, Santini F. Diagnosis and treatment of lipodystrophy: a step-by-step approach. J Endocrinol Invest. 2019 Jan;42(1):61-73.
2. Spartalis M, Spartalis E, Tzatzaki E, Tsilimigras DI, Moris D, Kontogiannis C, Kaminiotis VV, Paschou SA, Chatzidou S, Siasos G, Voudris V, Iliopoulos DC. The Beneficial Therapy with Colchicine for Atherosclerosis via Anti-inflammation and Decrease in Hypertriglyceridemia. Cardiovasc Hematol Agents Med Chem. 2018;16(2):74-80.
3. Boulanger, C.M. Endothelium. Arterioscler. Thromb. Vasc. Biol. (2016) 36, e26-e31.
4. Ooi, B. K., Chan, K. G., Goh, B. H., and Yap, W. H. The role of natural products in targeting cardiovascular diseases via Nrf2 pathway: novel molecular mechanisms and therapeutic approaches. Front. Pharmacol. (2018). 9,1308.
5. Barton M, Yanagisawa M. Endothelin: 30 Years From Discovery to Therapy. Hypertension. (2019) Dec;74(6):1232-1265.
6. Saida K, Mitsui Y, Ishida N. A novel peptide, vasoactive intestinal contractor, of a new (endothelin) peptide family. Molecular cloning, expression, and biological activity. J Biol Chem. 1989;264:14613-14616.
7. Abd-Elsalam MA. Bosentan, a selective and more potent antagonist for Atractaspis envenomation than the specific antivenom. Toxicon. 2011);57:861-870.)
8. Barton M, Yanagisawa M. Endothelin: 20 years from discovery to therapy. Can J Physiol Pharmacol. 2008;86:485-498
9. Rapoport RM, Zuccarello M. EndothelinA–EndothelinB Receptor Cross Talk in Endothelin-1–Induced Contraction of Smooth Muscle. Journal of cardiovascular pharmacology. 2012 Nov 1;60(5):483-94.
10. Lerman A, Edwards BS, Hallett JW, Heublein DM, Sandberg SM, Burnett JC Jr. Circulating and tissue endothelin immunoreactivity in advanced atherosclerosis. N Engl J Med. 1991;325:997–1001.
11. Ali H, Rustam R, Aprilia D, Arizal C, Gusadri IB, Utami PR. Upregulation of SCUBE2 expression in dyslipidemic type 2 diabetes mellitus is associated with endothelin-1. Diabetes Metab Syndr. (2019) Sep-Oct;13(5):2869-2872.
12. Tsikas D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. Anal Biochem. (2017),May 1;524:13-30.
13. Razygraev AV, Yushina AD, Titovich IA. A Method of Measuring Glutathione Peroxidase Activity in Murine Brain in Pharmacological Experiments. Bull Exp Biol Med. (2018).Jun; 165(2):292-295.
14. Didangelos T, Karlafti E, Kotzakioulafi E, Kontoninas Z, Margaritidis C, Giannoulaki P, Kantartzis K. Efficacy and Safety of the Combination of Superoxide Dismutase, Alpha Lipoic Acid, Vitamin B12, and Carnitine for 12 Months in Patients with Diabetic Neuropathy. Nutrients. (2020),Oct 23;12(11): 3254.
15. Bancroft, JD and Marilyn, G (2008). Theory and practice of histological techniques. 1st Edn., London, Elsevier Limited. PP: 168-173.
16. Echeverri I, Ortega-Avila JG, Mosquera M, Castillo A, Jimenez E, Suarez-Ortegon MF, et al. Relationship between maternal and newborn endothelial function and oxidative stress. Am J Hum Biol. (2015)
17. Campia U, Matuskey LA, Tesauro M, Cardillo C, Panza JA. PPARγ activation does not affect endothelin activity in non-diabetic patients with hypertension or hypercholesterolemia. Atherosclerosis. (2014).Jun;234(2):436-440.
18. Black SM, Fineman JR. Oxidative and nitrosative stress in pediatricvpulmonary hypertension: roles of endothelin-1 and nitricvoxide. VasculvPharmacol. (2006) 45:308-316.
19. Kambas K, Chrysanthopoulou A, Kourtzelis I, Skordala M, Mitroulis I, Rafail S, et al. Endothelin-1 signaling promotes fibrosis in vitro in a bronchopulmonary dysplasia model by activating the extrinsic coagulation cascade. J Immunol. (2011)
20. Khan MA and Baseer A. Increased malondialdehyde levels in coronary heart disease. J Pak Med Assoc (2000); 50: 261–264.