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Research Article

RAPAMYCIN PREVENTS OXIDATIVE STRESS AND LIPID ALTERATIONS IN ATHEROGENIC DIET FED C57BL/6J MICE

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Abstract

Dyslipidemia is the most important risk factor for atherosclerosis, which leads to the organ dysfunction and oxidative stress. In this study, we aimed to evaluate the effect of rapamycin on oxidative stress and lipid abnormalities in heart and kidney tissues. Atherogenic diet induced dyslipidemic mice model was used in this study. Rapamycin was treated once in a week for twelve weeks. Tissue angiotensin converting enzyme (ACE) activity and lipid composition was evaluated. Lipid peroxidation was assessed by the quantification of tissue thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH). The results of the study have shown that, atherogenic dyslipidemia elevates cardiac and renal tissue oxidative stress. Moreover it enhanced the activity of tissue ACE and affects the tissue lipid composition (elevated cholesterol, triglycerides, free fatty acids and reduced phospholipids). Atherogenic mice treated with rapamycin have shown to be brought back all the above changes to near normal. In conclusion, this study has proved that rapamycin have beneficial role on the cardiac and renal system in addition with its preventive effect on oxidative stress.

Keywords: Rapamycin; dyslipidemia; atherosclerosis; angiotensin converting enzyme

1.Introduction

Atherosclerosis, a major contributor to morbidity and mortality in developed countries, is the underlying cause of a number of cardiovascular diseases and is closely associated with dyslipidemia. Significant research has demonstrated that lipid-associated disorders, such as atherosclerosis, are linked to alterations in hemodynamic parameters, which may result in pathological cardiovascular events (Libby, 2006; Russell and Proctor, 2006).

Dyslipidemia is recognized as a prominent risk factor for cardiovascular (CV) disease (Yusuf et al., 2004). Atherogenic dyslipidemia comprises a triad of increased blood concentrations of small, dense low-density lipoprotein (LDL) particles, decreased high-density lipoprotein (HDL) particles, and increased triglycerides. A typical feature of obesity, metabolic syndrome, insulin resistance, and type 2 diabetes mellitus, atherogenic dyslipidemia has emerged as an important risk factor for myocardial infarction and cardiovascular disease (Musunuru, 2010). A previous study has shown that dyslipidemia impairs cellular remodelling in compensated cardiac hypertrophy accompanied by enhanced carbohydrate oxidation, cardiac lipid accumulation, and an exaggerated decline in contractile efficiency (Akki and Seymour, 2009).

In oxidative stress point of view previous evidences have shown that, feeding of high fat diet is accompanied by increased hepatic, heart, and renal tissue oxidative stress, which is characterized by reduction in the activity of antioxidant enzymes and glutathione levels, that correlate with the increase in lipid peroxidation level in most tissues (Noeman et al., 2011). Another study has demonstrated that the pathways for reactive oxygen species (ROS) production and oxidative stress are

coordinately up-regulated in tissues of dyslipidemic animals (Matsuzawa-Nagata et al., 2008). In antioxidant point of view, a previous study has shown that, C57BL/6 mice fed with high fat diet resulted in significant alterations in lipid profiles and a depressed antioxidant defence system and lipoic acid supplementation induces decrease in lipid peroxidation (Yang et al., 2008).

Rapamycin prolongs life span in heterozygous, inbred and cancer-prone mice (Leontieva et al., 2013). Previous study with similar treatment regime of rapamycin like the current study has suggested that it prevented the effect of the high-fat diet on the rate of accretion in body weight via reducing lipid accumulation (Chang et al., 2009). Atherosclerosis in C57BL/6J mouse strains has been widely studied by using an atherogenic (Ath) diet containing cholesterol, cholic acid and fat. By using this modified atherogenic (Ath) diet, Paigen et al. (1987) demonstrated that fatty streak lesion formation is reproducible within a strain, and that strains differ in their susceptibility. The C57BL/6J strain was among the most susceptible and has been extensively used as a model for diet induced atherosclerosis. In this study, we indented to evaluate the cardioprotective and antioxidant efficacy of rapamycin on cardiac and renal tissues in atherogenic diet fed dyslipidemic mice.

2. Materials and methods

2.1. Animals and chemicals

C57BL/6J mice were procured from NIN, Hyderabad, India for this study. This experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital, Annamalai nagar, Tamil Nadu, India. All other chemicals used in this study were of analytical grade obtained from Merck and Himedia, India.

2.2. Atherogenic diet and treatment schedule

Atherosclerosis was induced by feeding the mice with diet containing cholesterol (1.25%), fat (15%), and cholic acid (0.5%). Each of the following groups consisted of twelve animals. Rapamycin was injected to mice intraperitonially at the dose of 2mg/kg body weight, once a week for 16 weeks. This dose regime was followed as described in a previous study (Chang et al., 2009).

Group I - Control (fed with standard rodent chow)
Group II - Control + Rapamycin (2 mg/kg body weight)
Group III - Control animals fed with atherogenic diet (Ath)
Group IV - Ath + Rapamycin (2 mg/kg body weight)

2.3. Estimation of lipid peroxidation products

Heart and kidney tissues were sliced into pieces and homogenized in phosphate buffer in cold condition (pH 7.4) to give 20% homogenate (w/v). The homogenate was centrifuged at 560× g for 10 min at 4°C in refrigerated centrifuge. The supernatant was separated and used for various biochemical estimations. The level of thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) was estimated by the methods of Niehaus and Samuelson (1968), Jiang et al. (1992) respectively.

2.4. Estimation of heart and kidney lipids

Heart and kidney tissue lipids were extracted by the method of Folch et al. (1957). The sample was homogenized in cold chloroform-methanol (2:1 v/v) and the contents were extracted after 24 hours. The combined filtrate was washed with physiological saline and the aqueous layer was discarded. The lipid extract was re-dissolved in 3.0 mL of chloroform–methanol (2:1) mixture and aliquots were taken for the estimation of lipids. Total cholesterol (TC), triglycerides (TG), free fatty acids (FFA), and phospholipids (PL) were estimated by the methods of Allain et al. (1974), McGowan et al. (1983), Falholt et al. (1973) and Zilversmit and Davis (1950), respectively.

2.5. Determination of ACE activity

The angiotensin-converting enzyme (ACE) activity in heart and kidney tissues was measured by the spectrophotometric assay as described previously (Sharma et al., 2012). ACE activity in tissue homogenate was

measured by hydrolysis of Hip–His–Leu. Briefly, Hip–His–Leu was hydrolyzed into hippuric acid and His–Leu by angiotensin converting enzyme. Hippuric acid was extracted by ethyl acetate and determined at 228 nm. ACE activity was expressed as milliunit per mg protein.

2.6. Statistical analysis

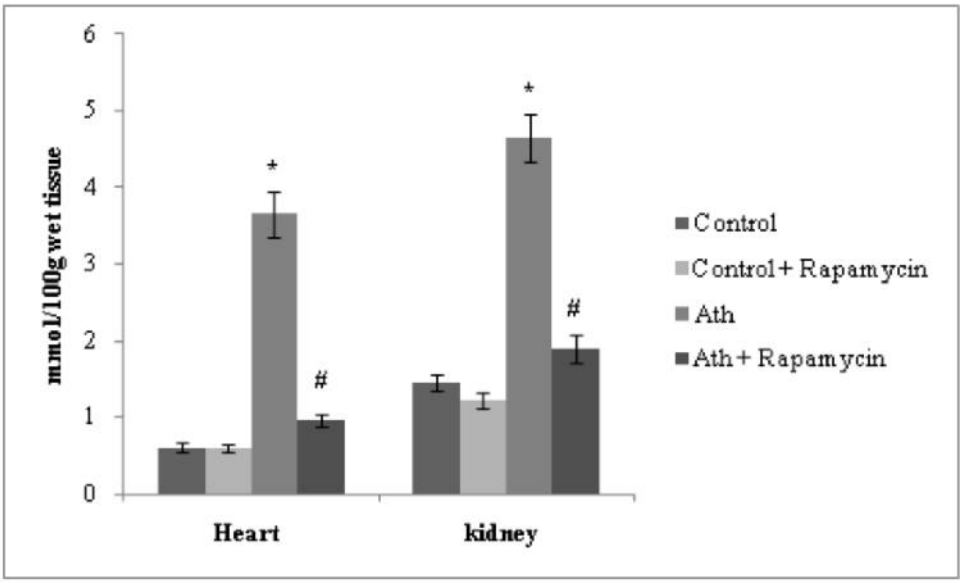
Values are given as means ± S.D. for six mice in each group. Data were analyzed by one-way analysis of variance followed by Duncan's multiple range test using SPSS version 11.5 (SPSS, Chicago, IL). The limit of statistical significance was set at $P < 0.05$.

3. Results

3.1. Lipid peroxidation

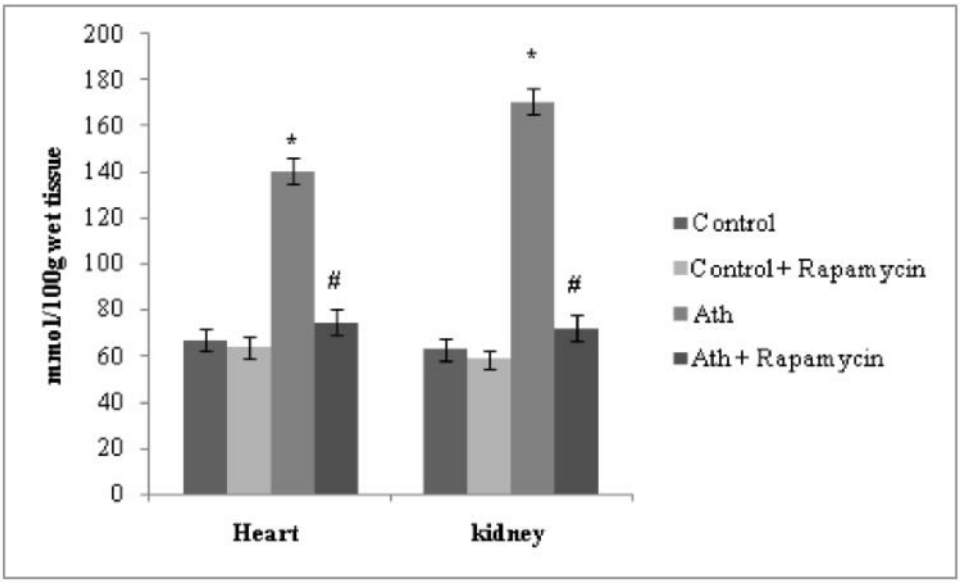
The lipid peroxidation markers TBARS and LOOH are the important indicators of oxidative stress. In this study atherogenic diet increased the formation of lipid peroxidation products in heart and kidney. Treatment with rapamycin significantly (P < 0.05) reduced the level of lipid peroxidation products (Fig. 1 and 2).

Fig. 1. Effect of rapamycin on TBARS.



Values are means ± S.D. for six mice. * differs significantly at P < 0.05 compared with control mice. # differs significantly at P < 0.05 compared with Ath mice (Duncan's multiple range test).

Fig. 2. Effect of rapamycin on lipid hydroperoxides.



Values are means ± S.D. for six mice. * differs significantly at P < 0.05 compared with control mice. # differs significantly at P < 0.05 compared with Ath mice (Duncan's multiple range test).

3.2. Cardiac and renal lipids

Atherogenic diet fed mice shows significant increase in the levels of TC, TG and FFA with a significant decrease in PL. On treatment with rapamycin, the levels of all the above parameters brought to near normal level (Tables 1 and 2)

Table 1 Effect of rapamycin on cardiac lipids

Groups	TC	TG	PL	FFA
Control	2.59 ± 0.15	3.58 ± 0.22	12.56 ± 0.97	4.54 ± 0.33
Control + Rapamycin	2.68 ± 0.12	3.63 ± 0.19	11.92 ± 0.84	4.14 ± 0.27
Ath	6.32 ± 0.49*	6.95 ± 0.51*	7.95 ± 0.56*	6.34 ± 0.48*
Ath+ Rapamycin	3.87 ± 0.27#	4.86 ± 0.32#	10.08 ± 0.81#	5.11± 0.42#

Values are means ± S.D. for six mice. * differs significantly at $P < 0.05$ compared with control mice. # differs significantly at $P < 0.05$ compared with Ath mice. (Duncan's multiple range test).

Table 2 Effect of rapamycin on renal lipids

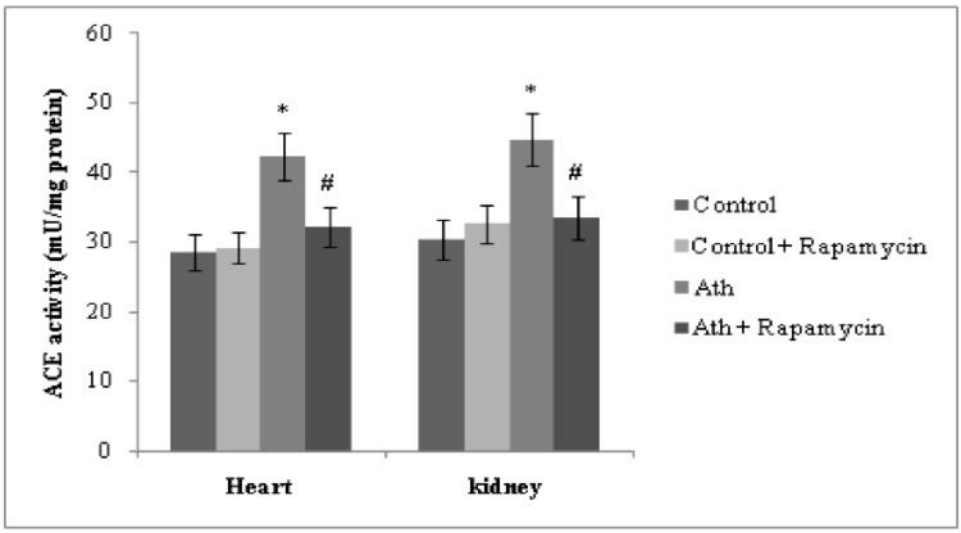
Groups	TC	TG	PL	FFA
Control	3.72 ± 0.23	4.87 ± 0.35	14.33 ± 0.85	3.96 ± 0.28
Control + Rapamycin	3.86 ± 0.27	4.69 ± 0.23	13.97 ± 0.76	3.74 ± 0.25
Ath	7.59 ± 0.53*	7.87 ± 0.55*	9.15 ± 0.65*	7.89 ± 0.58*
Ath + Rapamycin	4.51 ± 0.31#	5.08 ± 0.36#	12.11± 0.61#	4.83 ± 0.37#

Values are means ± S.D. for six mice. * differs significantly at $P < 0.05$ compared with control mice. # differs significantly at $P < 0.05$ compared with Ath mice. (Duncan's multiple range test).

3.3. ACE activity

Fig. 3 depicts the ACE activity in heart and kidney tissues. Atherogenic diet significantly ($P < 0.05$) enhanced the activity of ACE in heart and kidney compared with control whereas rapamycin treatment significantly reduces the ACE activity.

Fig. 3. Effect of rapamycin on ACE activity.



Values are means ± S.D. for six mice. * differs significantly at $P < 0.05$ compared with control mice. # differs significantly at $P < 0.05$ compared with Ath mice (Duncan's multiple range test).

4. Discussion

Dyslipidemia is associated with accelerated macrovascular and microvascular coronary disease and cardiomyopathic phenomena, inhibition of ACE activity can attenuate all the above (Zaman et al., 2001). A study has previously shown that inhibition of ACE activity prevents coronary perimicrovascular fibrosis in genetically obese mice that develop insulin resistance (Zaman et al., 2004). In this study, atherogenic diet fed mice shows increased ACE activity and rapamycin treatment reduce the activity in both heart and kidney tissues. When focused on kidney, previous study in diabetic atherogenic model has shown that reducing ACE activity is beneficial, in which both the local renal ACE inhibition and superior antihypertensive effect of omapatrilat contribute to its renoprotective effect (Jandeleit-Dahm et al., 2005). Thus rapamycin treatment has sown its protective role on heart by reducing ACE activity and it extends its beneficial effect on kidney.

Lipids were susceptible to oxidation, lipid peroxidation products are potential biomarkers for oxidative stress status *in vivo* and its related diseases (Niki, 2008). In this study the lipid peroxidation products were increased in atherogenic diet fed mice heart. A previous study indicates that lipid radicals react with oxygen to form lipid peroxy radicals, which in turn react and transfer electrons with neighbouring lipids and initiating a chain reaction that destroys the integrity of the membrane. It was previously known that Western diet impairs metabolic remodelling and contractile efficiency in cardiac hypertrophy (Akki and Seymour, 2009). Lipid peroxidation affects membrane permeability and alters membrane bound enzymes/ion channels, which disturbs ion transport lead to Ca^{2+} overload and demonstrates direct cardiac depression by malondialdehyde at the ventricular myocyte level, possibly through oxidative stress (Folden et al., 2003). These dysfunctional responses may result from the redox modification of proteins involved in excitation-contraction coupling and/or mitochondrial energy production (Shenouda et al., 2008). In this study, the result shows that rapamycin treatment reduces the lipid peroxidation and protects the cardiac cells from lipid peroxidative damage.

Lipid compositional alteration in heart and kidney was found in this study. Enhanced accumulation of cholesterol and triglycerides in heart and kidney was observed. It was previously known that atherogenic diet induces dyslipidemia and elevates low-density lipoprotein (LDL) level (Paigen et al., 1987). The observed increase in the myocardial cholesterol content in atherogenic mice is due to increased uptake of LDL-cholesterol from the blood by myocardial membranes. Another study has explained that cardiomyocyte triglyceride accumulation and reduced ventricular function in mice (Ge et al., 2012). In a number of pathophysiological states, there is evidence to support that a mismatch between myocardial uptake and oxidation of fatty acids may lead to abnormally high intracellular triglyceride levels and contractile dysfunction. Furthermore, previous study has shown that, cardiac lipid accretion and contractile dysfunction is associated with altered expression of metabolic genes (Akki and Seymour, 2009). In this study the phospholipid composition also reduced in heart and kidney. A significant decrease in phospholipid content could be due to an accelerated degradation of membrane phospholipids by phospholipases (Farber and Young, 1981). Rapamycin treatment prevents the level of above lipid changes in coordination with reducing lipid peroxidation in cardiac membranes and kidney tissue thereby it improves cardiac function and protect the renal changes.

In conclusion, the overall study have explored that, rapamycin treatment have beneficial role on atherogenic condition induced oxidative stress, ACE activity and tissue lipid abnormalities. In future, after complete validation we will reposition rapamycin against atherosclerosis related cardiovascular and renal dysfunctions.

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