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by Irsan Saleh5

Telmisartan Prevents Myocardial Fibrosis via Decreasing Fraction of Colagen Type 1 Volume in Myocardial Tissue in Wistar Rats-Induced High Salt Intake

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Abstract

Myocardial fibrosis is a pathological condition that responsible for initiation of heart failure. Neurohormonal endogen, angiotensin II, has a potential role to activate endothelin I, TGF-β1, myocardial fibroblast, extracelullar matrix deposition, structural changes and decreasing of cardiac function. Fibrotic process is also influenced by PPAR γ. Telmisartan has a potential effect to inactivate angiotensinergic system and to activate PPAR γ. It is expected that telmisartan has optimal effect to protect myocardial fibrosis. To know the role of variation dose of telmisartan to decrease collagen type 1 fraction volume in cardiac tissue of Wistar rats.

Ten-week-old male Wistar Rat (n = 30) were randomized into five groups, and each group consisted of 6 rats. Group 1 : negative control. Group 2 : rats were induced by intake Nacl 8% doses 2% body weight for eight weeks. Group 3 : rats were induced by intake Nacl 8% doses 2% body weight and telmisartan 3 mg/kgBB for eight weeks. Group 4 : rats were induced by intake Nacl 8% doses 2% body weight and telmisartan 6 mg/kgBB for eight weeks. Group 5 : rats were induced by intake Nacl 8% doses 2% body weight and telmisartan 12 mg/kgBB for eight weeks. Collagen volume fraction was assessed by immunohistochemistry and ImageJ program. ANOVA test followed pos hoc test was used to analyzed each variable.

Collagen volume fraction significantly decreased in group 3, 4 and 5 compared in group 2. Telmisartan decreases collagen type 1 volume fraction of myocardial tissue .

Keywords : Telmisartan-Myocardial Fibrosis-Colagen Type 1-Rat-High Salt

Introduction

Myocardial fibrosis is a pathological condition that underlies the occurrence of heart failure.[1-4] Endogenous neurohormonal, angiotensin II, plays a role in endothelin activation I and transforming growth factor-β1 (TGF-β1), leading to the activation of myocardial fibroblasts. Myocardial fibroblasts will produce collagen which will form an

extracellular matrix so that there will be extracellular matrix deposition, structural changes and decreased heart function.[5-11]

The fibrosis process is also affected by the peroxisome proliferator activated receptor (PPAR) subunit γ. Some lipid derivatives, particularly unsaturated fatty acids, are ligands of this receptor.[12,13]

PPAR γ is expressed primarily on adipose tissue, which plays a role in improving insulin sensitivity. PPAR γ agonists are clinically used in the management of diabetes mellitus and metabolic syndrome.[14] PPAR γ is also found in various organs including the heart and kidneys.[15,16] PPAR γ plays a role in cellular differentiation, tumor cell antiproliferation activity and miofibroblasts.[17] Activation of PPAR γ will Prevents bonding between the p-300 protein and the Smad protein, thus inhibiting the activation of myocardial fibroblasts.[18] In addition, PPAR γ will prevent the association of the Smad3 protein (R-Smad) with response element and Co-Smad protein and thus inhibit the production of TGF-β1.[19]

Telmisartan is an angiotensin I receptor blocker (ARB) which is useful as an anti-hypertensive drug and is identified as partial agonist PPAR γ .[20,21] Related to activity on PPAR γ, telmisartan is believed to play a role in inflammatory response, malignancy management and management of cardiovascular disorders. This study aims to look at myocardial cardioprotective effects associated with myocardial fibrosis by assessing the fraction of collagen type 1 volume in the hearts of wistar rats.

Methods

Male Wistar rats, 10 weeks of age and body weight between 150-200g, were obtained from Laboratorium Penelitian dan Pengujian Terpadu (LPPT) Universitas Gadjah Mada (UGM) Yogyakarta. This research activity has received ethical approval from LPPT UGM ethics committee. Male Wistar rats (n = 30) performed randomization into five groups, each group of 6 rats. Group 1: negative control. Group 2: NaCl 8% given 2% weight for 8 weeks and aquades 5 ml. Group 3: NaCl 8% given dose 2% body weight and telmisartan 3 mg / kgBW, group 4: given 8% NaCl 2% dose 2% body weight and telmisartan 6 mg / kgBW and group 5: given 8% NaCl 2% dose body weight And telmisartan 12 mg / kgBW for 8 weeks. Every week, systolic blood pressure is measured with tail-cuff detection .[22]

Rats performed anesthesized with ketamine (75mg / kgBB intraperitoneal). The organs of the heart was immediately isolated from the chest cavity and left ventricular heart removal was performed. The left ventricle was inserted in a 10% formalin solution for examination of collagen volume fractions.[22] The tissue was made of paraffin block preparations. Made of 5 μm thick slices, deparafinization, peroxidase blocking, retrieval antigen, non-specific binding blocking, IHC staining proper with anti-collagen type 1 antibody, staining with DAB, counterstain, dehydration and mounting. Collagen volume fraction was assessed with the help of the imageJ program. Each preparation preparation taken 15 field of view with magnification 40x.[22]

The results of the analysis were shown as mean ± SEM. Systolic blood pressure, weight and heart weight were compared between groups with ANOVA assay followed by post hoc (bonferroni test). The mean of collagen volume fraction was compared between groups with ANOVA assay followed by post hoc (Bonferroni test). The difference was statistically significant when $p < 0.05$.

Results

Heart Weight

Heart weight showed significant differences between treatment groups (Group 1: 590 ± 1.78; Group 2: 648 ± 0.98; Group 3: 641 ± 1.50; Group 4: 636 ± 1.91; Group 5: 612 ± 1.25; p = 0.00). As shown in table 1.

Table 1. Heart Weight

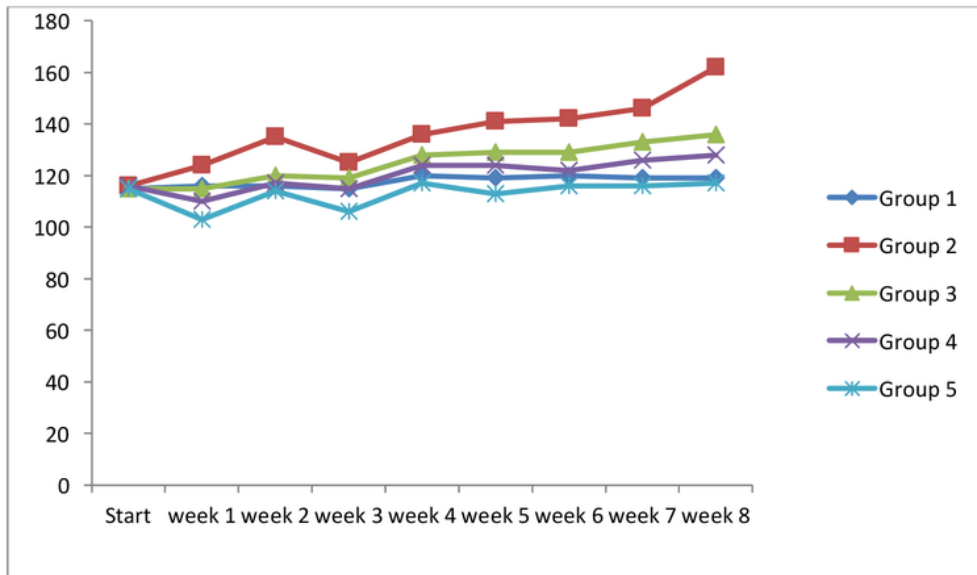| No.	Group	Heart Weight (mg/100 gram BW)* (Mean ± SEM)
1.	1	590 ± 1,78
2.	2	648 ± 0,98
3.	3	641 ± 1,50
4.	4	636 ± 1,91
5.	5	612 ± 1, 25

* P <0.05 ANOVA test

Systolic Blood Pressure

Systolic blood pressure before treatment showed no significant difference between groups (Graph 1). After 8 weeks treatment, systolic blood pressure did not show a significant increase in group 1 (negative control), but increased in the other group. Increased blood pressure significantly occurred in group 2 who received high intake of sodium compared to other groups. Telmisartan significantly lowered systolic blood pressure in groups of 3, 4 and 5 when compared to group 2 (p = 0.00).

Table 2. Systolic Blood Preassure

group	Systolic Blood Preassure (mmHg) (Mean ± SEM)								
	start	Week I	Week II	Week III	Week IV	Week V	Week VI	Week VII	Week VIII
1	115.83±0,90	116±1,05	116,29±0,8	115,62±0,8	120,92±0,3	119,33±0,6	120,13±0,5	119,71±0,4	119,88±0,28
2	116±0,59	124,75±1,06	135,33±1,6	125,50±0,6	136,50±3,4	141,62±3,23	142,50±3,07	146,67±2,47	162,75±2,27
3	115,79±0,74	115,33±0,56	120,75±0,6	119,75±0,4	128,62±0,9	129,96±0,78	129,46±1,76	133,17±1,12	136,71±1,01
4	116,54±1,18	110,42±0,62	117,79±0,5	115,62±1,2	124,92±1,7	124,29±0,86	122,54±0,91	126,16±0,55	128,71±0,56
5	115,45±1,67	103,1±1,20	114,58±0,9	106,25±1,2	117,46±1,2	113,12±1,99	116,17±1,50	116,67±2,23	117,21±2,00

Figure 1. Systolic Blood Preassure

Fraction of Collagen Type 1 Volume

Table 3 showed the mean fraction of collagen volume of experimental animals of group 1,2,3,4 and group 5. Based on one-way ANOVA test there was a significant difference between group 1,2,3,4 and group 5. Post hoc test, A significant difference between group 1 and group 2, $p = 0.00$. These results indicate an average difference in fraction volume of collagen between groups not receiving 8% sodium chloride supplementation and 8% sodium chloride group. Based on post hoc test, there were significant differences between group 2 and group 3.4 and 5. These results showed that there was a significant difference between the telmisartan and telmisartan group of 3 mg / kgBW, 6 mg / kgBW and 12 mg / KgBW.

Table 3. Fraction of Colagen Type 1 Volume

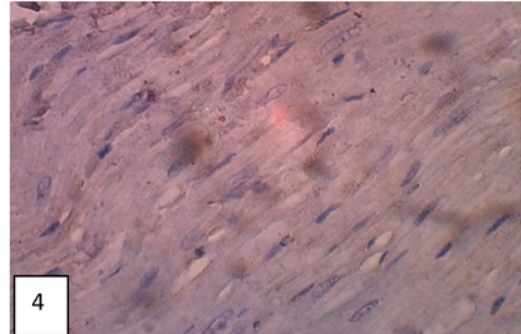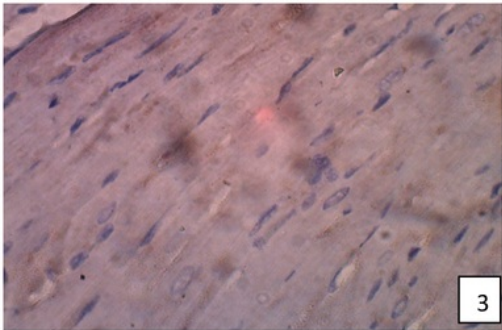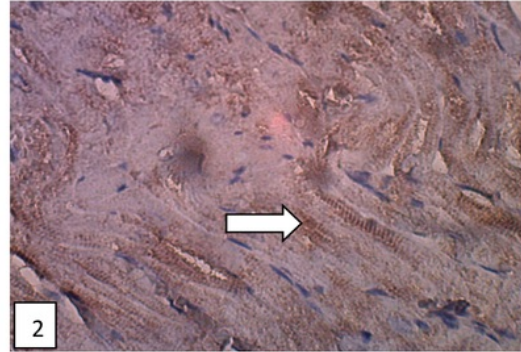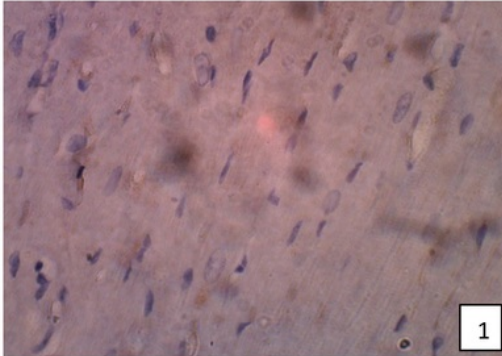| No.	Group	Fraction of Colagen Volume (%) (Mean ± SEM)
1.	1	$30,05 \pm 0,19$
2.	2	$34,45 \pm 0,57$
3.	3	$30,22 \pm 0,79$
4.	4	$26,24 \pm 0,56$
5.	5	$19,47 \pm 1,38$
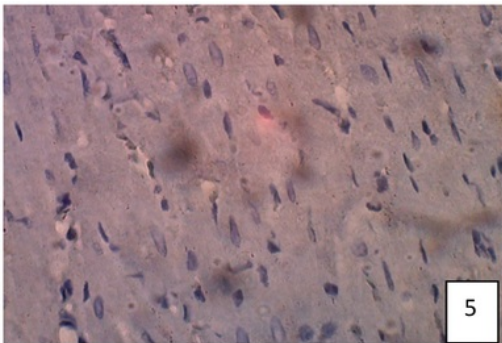
figure 2. Immunohistochemistry of Collagen Type 1 in Myocardial Tissue (400x)

1: group of animals that did not receive sodium chloride or telmisartan intake. 2: group of animals receiving sodium chloride 8% dose 2% animal body weight and aquadest 5 mL. 3: group of animals receiving sodium chloride 8% dose 2% body weight and telmisartan dose 3 mg / kgBW. 4: group of animals receiving sodium chloride 8% dose 2% body weight and telmisartan dose 6 mg / kgBW. 5: group of animals receiving sodium chloride 8% dose 2% body weight and telmisartan dose 12 mg / kgBW. White arrow : Collagen Type I

Discussion

(High salt intake) sodium chloride 8% caused increasing in systolic blood pressure and caused myocardial fibrosis. Sodium chloride 8% administration will initiate the occurrence of myocardial fibrosis and kidney glomerolus, which is associated with increased expression of TGF-β1.[22] Telmisartan is an ARB class of antihypertensive drugs (angiotensin-I Receptor Blocker) and has been identified as ligands of PPAR (peroxisome proliferator activator receptor) γ.[3] PPAR (peroxisome proliferator activator receptor) will lead to the formation of the complex PPAR, RE and NCoR that would inhibit TGF-β1.[23-26] The expression of PPAR γ will also prevent the binding between the protein p-300 with Smad proteins, thus preventing the recruitment of transcription factors that play a role in the activation miofibroblast. [27-33] Miofibroblas has a major role in the process of accumulation of collagen.

This study showed that administration of telmisartan dose of 3 mg / kg, 6 mg / kg and 12 mg / kg body weight may prevent the accumulation of collagen in the myocardial tissue in animals that received sodium chloride 8%. The results of this study are in accordance with the research which states that telmisartan may slow the occurrence of fibrosis in the heart of rats with left ventricular hypertrophy.[34, 35] The role of telmisartan administration a dose of 3 mg / kg, 6 mg / kg and 12 mg / kg in lowering the fraction of collagen volume and accumulation of collagen in the myocardial tissue.

Conclusion

Collagen volume fraction significantly decreased in group 3, 4 and 5 compared in group 2. Telmisartan decreases collagen type 1 volume fraction of myocardial tissue .

Acknowledgments

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