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Original Article

Telmisartan Prevents Myocardial Fibrosis : Telmisartan Decreases TGF-β1 and Collagen Fraction Volume Myocardial Tissue in Wistar Rats Induced High Salt Intake

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ABSTRACT

The beneficial effects from variation doses of telmisartan prevents myocardial fibrosis in wistar rats induced high salt intake.Ten-week-old male Wistar Rat (n = 30) were used. Wistar Rats were randomized into five groups. Group 1 : 6 rats as negatif control. Group 2 : 6 rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight for eight weeks. Group 3 : 6 rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 3 mg/kgBB for eight weeks. Group 4 : 6 rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 6 mg/kgBB for eight weeks. Group 5 : 6 rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 12 mg/kgBB for eight weeks. Fraction volume collagen and concentration of TGF-β1 in myocardial tissue were assessed. Fraction volume collagen and TGF- β1 significantly decreased in group 4 (high salt intake and telmisartan 6 mg/kgBB) and 5 (high salt intake and telmisartan 12 mg/kgBB) compared in group 2 (only high salt intake).Telmisartan prevents myocardial fibrosis. Telmisartan decreases TGF-β1 and fraction volume collagen in myocardial tissue wistar rats induced high salt intake.

Introduction

Congestive heart failure (CHF) is mainly resulted from fibrosis heart. Accumulation collagen in heart fibrosis occurs as result from activity of myofibroblast [1-4]. Myofibroblast is throught to be driven by extracellular stimuli, transformation growth factor β1 (TGF-β1) is one of major player. Interestingly, if the surrounding pathogenic factors are removed, myofibroblast has potential possibility to be reversed [5-11]. Beyond delaying the inevitable onset of CHF cessation and regression of myofibroblast are ideal schemes of clinical treatment. Realizing the process of myofibroblast is essential in establishing therapeutic strategies for progressive CHF, the pathway to counteract and reverse myofibroblast seems to be focused.

Peroxisome proliferators-activated receptors (PPARs) are members of nuclear receptor superfamily with 3 isotypes existed in mammals: PPARα, PPARβ and PPAR-γ . Steroid, thyroid and retinoid hormones are ligands for the receptors. PPAR-γ is highly expressed in adipose tissue; its activation plays a key role in increasing systemic insulin sensitivity [12,13]. PPAR-γ agonists are clinically used in the treatment of type 2 diabetes mellitus and metabolic syndrome [14]. PPAR-γ has been found constitutively expressed in many other tissues like in kidney and heart. It has been thought to play a protective role for the organ [15,16]. Also PPAR-γ expression in macrophages and lymphocytes suppresses inflammatory responses and PPAR-γ agonists inhibit the production of pro inflammatory cytokines and regulate the process of inflammation by activating this nuclear receptor[17]. More interested, PPAR-γ mediates cellular differentiation, antiproliferative activity in various tumor types and myofibroblast [18]. A number of data support a direct role of PPARs in modulating Smad transcriptional complex binding downstream of TGF-β signaling. The idea that PPAR inhibits Smad transcriptional activity by trans-repression mechanisms is supported by reports of PPARγ-mediated inhibition of the p300-Smad2/3 association [19].

Telmisartan is a highly selective angiotensin II type 1 (AT1) receptor blocker (ARB) for treatment of cardiovascular diseases as hypertension. In 2004, it was first identified Telmisartan as partial agonist of PPAR-γ in treatment of hypertension and diabetes [20,21]. Later on, Telmisartan was reported to connected its functions with PPAR-γ in inflammation, cancer treatment , as well as used for treating patients with CHF [18]. Now, we address the question whether Telmisartan has the capacity of reversing the process of myofibroblast in myocardial to counteract myofibroblast-related pathological change as fibrosis heart by lowered TGF-β1 via PPAR-γ pathway.

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MATERIALS AND METHODS

This study was carried out in strict accordance with the recommendations in the guide for care and use of laboratory animals of research laboratory Gadjah Mada University. The protocol was approved by the Committee on the Ethics of Animal Experiment Research Laboratory Gadjah Mada University.

Experimental Protocol :

Ten-week-old male Wistar Rat (n = 30) were used. Wistar Rats were randomized into five groups. Group 1 : 6 rats as negatif control. Group 2 : 6 rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight for eight weeks. Group 3 : 6 rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 3 mg/kgBB for eight weeks. Group 4 : 6 rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 6 mg/kgBB for eight weeks. Group 5 : 6 rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 12 mg/kgBB for eight weeks. Every week, systolic blood preassure were measured by tail-cuff technique [22].

Tissue Collection :

Rats were anasthezied by ketamin 75 mg/kgBB intraperitoneal and killed by decapitation. The hearts were rapidly excised. The hearts were divided at coronary plane at its ventricular equator, and the upper half were fixed in 10% formalin solution for fraction volume collagen analysis. The lower half were fixed in liquid nitrogen for TGF-β1 analysis [22].

TGF-β1 Analysis :

Sample were washed by PBS 1% for five times. Every sample was crashed by mortar, add 0,5 mL sample buffer and centrifuge 10.000 rpm for 10 minutes. Supernatan was collected. Solid phase sandwich ELISA (Rat TGF-β1 ELISA kit, Boster) were used to analysis concentration of TGF-β1. Add samples and standards and incubate the plate at 37°C for 90 minutes, do not wash. Add biotinylated antibodies and incubate the plate at 37°C for 60 minutes, wash plate 3 times with PBS 0,1%. Add ABC working solution and incubate the plate at 37°C for 30 minutes. Wash plate 5 times with PBS 0,1%. Add TMB color developing agent and incubate the plate at 37°C in dark for 20 minutes. Add TMB stop solution and read the OD absorbance at 450nm in a microplate reader. The standard curve was plotted as the OD 450 of each standard solution vs the concentration of standard solution. The rat TGF-β1 concentration of the samples was interpolated from the standard curve.

Collagen Fraction Volume Analysis :

Sections 5μm thick were deparaffinazed, rehydrated, and then stained with 0,1% sirrius red in saturated picric acid (picrosirius red) for 1 hour. Fraction volume collagen were determined by measuring the area of stained tissue within a given field. The area stained were calculated as a percentage of total area within a field by programme Image J. Within the heart, fields containing vessels, artifact, minor scars were excluded. In left ventricle, 20-25 fields were analyzed [22].

Statistics :

The results are presented as mean ± SE. Systolic blood preassure and body weight were compared among group by ANOVA followed by post hoc analysis with bonferroni test. The mean concentartion of TGF- β1 and mean fraction volume collagen were compared among groups by Independent Student T-Test. A value of p<0,05 was considered statistically significant.

RESULT :

Body Weight :

Gained body weight was different among the groups (Group 1: 36.81 ± 1.6 ; Group 2: 48.57 ± 3.13; Group 3: 37.84 ± 4.03 ; Group 4: 44.47 ± 3.54 ; Group 5: 63.38 ± 4.11 ; p= 0.00). All groups gained weight over the experimental period.

Table 1. Mean of Body Weight of Rats Before and After Experimental Period

Group	Before (gram) (Mean ± SEM)	After (gram) (Mean ± SEM)	Gained Weight* (gram) (Mean ± SEM)
1	196.58 ± 1.8	233.39 ± 0.3	36.81 ± 1.6
2	183.40 ± 3.8	231.97 ± 1.9	48.57 ± 3.13
3	187.84 ± 3.2	225.25 ± 1.1	37.41 ± 4.03
4	183.28 ± 3.29	227.75 ± 2.7	44.47 ± 3.54
5	166.90 ± 3.28	230.28 ± 2.1	63.38 ± 4.11

***P<0.05 ANOVA test**

Group 1 : negatif control. Group 2 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight for eight weeks. Group 3 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 3 mg/kgBB for eight weeks. Group 4 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 6 mg/kgBB for eight weeks. Group 5 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 12 mg/kgBB for eight weeks.

Heart Weight :

Heart weight was not similar among the 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).

Table 2. Heart Weight

Group	Heart Weight (mg/100 gram body weight)* (Mean ± SEM)
1	590 ± 1.78
2	648 ± 0.98
3	641 ± 1.50
4	636 ± 1.91
5	612 ± 1.25

***P<0.05 ANOVA test**

Group 1 : negatif control. Group 2 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight for eight weeks. Group 3 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 3 mg/kgBB for eight weeks. Group 4 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 6 mg/kgBB for eight weeks. Group 5 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 12 mg/kgBB for eight weeks.

Systolic Blood Pressure

Systolic blood pressure at 10 weeks of age was not different among the groups. Over the study period, systolic blood pressure did not rise in group 1 (negative control), but increased in other groups. At 18 weeks of age, systolic blood pressure significantly increased in group 2 on high salt intake compared with other groups. Telmisartan significantly decreased systolic blood pressure in group 3,4 and 5 on high salt intake compared with group 2 on high salt intake without telmisartan (p= 0.00).

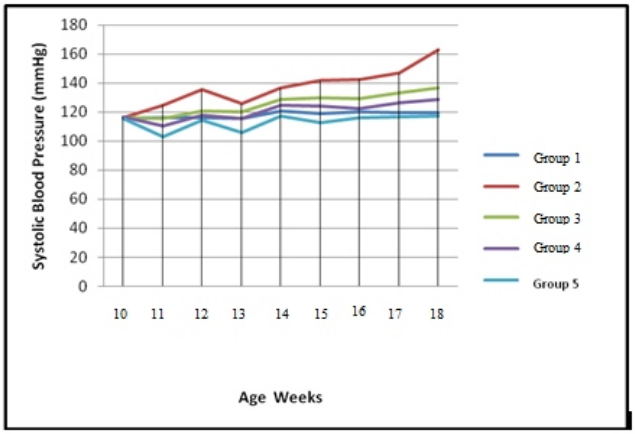

Figure 1. Change of blood pressure in rats from 10 to 18 weeks of age.

Concentration of TGF-β1 in Myocardial Tissue

High salt intake significantly rose TGF-β1 in myocardial tissue wistar rats group 2 compared in myocardial tissue rats group 1 without high salt intake (p=0.028). TGF-β1 concentration significantly decreased in group 4 (high salt intake and telmisartan 6 mg/kgBB) and 5 (high salt intake and telmisartan 12 mg/kgBB) compared in group 2 (only high salt intake) (p=0.00). TGF-β1 concentration did not different significantly in group 3 (high salt intake and telmisartan 3 mg/kgBB) compared in group 2 (only high salt intake) (p=0.078).

Table 3. TGF-β1 Concentration in Myocardial Tissue (ELISA)

Group	Concentration TGF-β1 (pg/mL)* (Mean ± SEM)
1	970, 84 ± 154,68
2	1354,60 ± 54,49
3	1173,80 ± 101,17
4	659,36 ± 74,51
5	492,73 ± 29,25

*** P<0.05, ANOVA test**

Group 1 : negatif control. Group 2 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight for eight weeks. Group 3 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 3 mg/kgBB for eight weeks. Group 4 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 6 mg/kgBB for eight weeks. Group 5 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 12 mg/kgBB for eight weeks.

Collagen Fraction Volume in Myocardial Tissue

High salt intake significantly increased fraction volume collagen in myocardial tissue wistar rats group 2 compared in myocardial tissue wistar rats group 1 without high salt intake (p=0.00). Fraction volume collagen significantly decreased in group 3 (high salt intake and telmisartan 3 mg/kgBB), group 4 (high salt intake and telmisartan 6 mg/kgBB) and 5 (high salt intake and telmisartan 12 mg/kgBB) compared in group 2 (only high salt intake) (p=0.00).

Collagen Fraction Volume in Myocardial Tissue

Group	Fraction Volume Collagen (%)* (Mean ± SEM)
1	12.53 ± 0.11
2	19.20 ± 0.13
3	16.35 ± 0.25
4	14.97 ± 0.38
5	13.21 ± 0.29

*** P<0,05**

Group 1 : negatif control. Group 2 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight for eight weeks. Group 3 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 3 mg/kgBB for eight weeks. Group 4 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 6 mg/kgBB for eight weeks. Group 5 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 12 mg/kgBB for eight weeks.

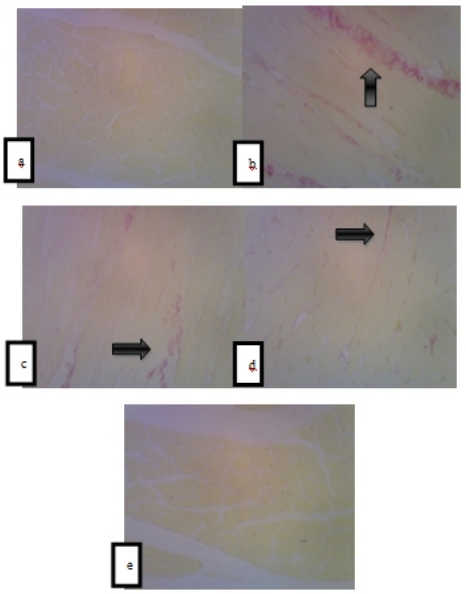

Figure 2. Picrosirius red staining of myocardial tissue under light microscopy (magnification x 40). a. Group 1 : negatif control. b. Group 2 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight for eight weeks. c.Group 3 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 3 mg/kgBB for eight weeks. d .Group 4 : rats were induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 6 mg/kgBB for eight weeks. e. Group 5 : rats were

induced for heart fibrosis by intake Nacl 8% doses 2% body weight and telmisartan 12 mg/kgBB for eight weeks. Black arrow shows crisscrosing of collagen network in group 2.

DISCUSSION :

This results of this study have important implications that high salt intake was confirmed to raise blood pressure and cause fibrosis myocardial. Furthermore, some studies have shown that high salt intake (8%) promotes fibrosis in myocardial tissue, intramyocardial arteries and kidney. These changes were associated with overexpression of TGF-β1 [22]. High salt intake (8%) for 8 weeks could be model for inducing fibrosis myocardial in wistar rats.

Telmisartan is a highly selective angiotensin II type 1 (AT1) receptor blocker (ARB) for treatment of cardiovascular diseases as hypertension. In 2004, it was first identified Telmisartan as partial agonist of PPAR-γ in treatment of hypertension and diabetes [20,21]. In the present study, we confirmed the efficacy of telmisartan prevents myocardial fibrosis in wistar rats induced high-salt intake via lowering TGF-β1. Telmisartan 12 mg/kgBB was more decrease TGF-β1 and fraction volume collagen in myocardial tissue than telmisartan 3 mg/kgBB and telmisartan 6 mg/kgBB (dose-dependance relationship).

Some studies support a direct role of PPARs in modulating Smad transcriptional complex binding downstream of TGF-β1 signaling. The idea that PPAR inhibits Smad transcriptional activity by trans-repression mechanisms is supported by reports of PPARγ-mediated inhibition of the p300-Smad2/3 association. In fact, ligand PPAR γ inhibited p300 recruitment by phospho-Smad2 or phospho-Smad3 on TGF- β1 treatment of cultured hepatocytes [23-26]. It should be noted that, under these conditions, the PPAR γ ligand affected neither the Smad2/3 protein level nor Smad2/3 phosphorylation. A likely explanation is that activation of PPARγ triggered its association with p300, a known NR co-activator [27-29], that then became limiting for Smad-mediated transcriptional regulation. A similar situation has been described in neonatal skin fibroblasts where PPAR γ agonists inhibited TGF β1-induced stimulation of collagen synthesis. Here also, this inhibition was correlated with inhibition of the formation of the complex between phopho Smad 1/2/3 and p300, which is PPAR γ dependent. Moreover, the trans-repression hypothesis was further supported in this case since it was shown that p300 overexpression could rescue collagen synthesis in the presence of a PPAR γ agonist [30-32].

CONCLUSION :

Telmisartan decreases concentration of TGF-β1 and collagen volume fraction of myocardial tissue wistar rats induced by high salt intake.

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