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The Effect of Quercetin for Salt Induces Renal Fibrosis by Suppressing Collagen Production

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Abstract- **The bad effect of salt have been suspected for a least one hundred years. High salt intake increased production of TGF-β whereas TGF-β activates a receptor serine/threonine kinase (RS/TK). Through phosphorylation Smad2/3 and Co-Smad cascade, they recruited transcription factors and co-activator for initiates gene transcription for cell proliferation, cell differentiation, and extramatriks cellular production include collagen. This mechanism initiates renal fibrosis. This study was quasi-experimental research with post-test only group design. A subject was 24 male Wistar rat induced with two %/kgBB doses of NaCl 8%. The subjects were divided into six groups. NaCl 8% and quercetin were delivered by gavage daily for eight weeks. After treatment the kidneys were taken for quantification the collagen volume fraction were stained with picrosirius red then quantification by Image J software. The level of Glomerular volume collagen fraction group OO (3.78±0.12 %), NO (19.05±0.99 %), NK5 (19.42±3.19 %) NK10 (17.14±0.70 %) and NK20 (3.22±0.81). Tubular volume collagen fraction group OO (2.26±0.08 %), NO (9.79±0.53 %), NK5 (9.72±0.98 %), NK10 (5.25±0.49 %), NK20 (1.92±0.20 %). Our data indicate that quercetin may be suppressing the collage volume fraction with a significant difference in the dose of 20 mg/kg BB quercetin.**

Keyword: quercetin, TGF-β1, collagen volume fraction, NaCl, renal fibrosis.

I. INTRODUCTION

Fibrosis is caused by the formation of excessive scar tissue in the organs, especially the lungs, blood vessels, heart and kidneys [1]. In the United States fibrosis associated with approximately 45% mortality [2]. Renal fibrosis is characterized by excessive scarring due to excessive production, deposition, and contraction of the extracellular matrix. The process of formation of fibrosis occurs over many months and years. Renal fibrosis is a significant cause of CKD (Chronic Kidney Disease) ended with ESRD (End Stage Renal Disease) can lead to organ dysfunction or death. The ESRD patient must do organ transplantation to improve the condition, but patients often die before take a suitable organ [3-6].

Preliminary research showed that 8% NaCl supplementation in Wistar Kyoto Rats for eight weeks increased the levels of TGF-β1 and collagen volume fraction in the kidney, left ventricle, and arterial intramyocardial without any significant increase in blood pressure [7].

Quercetin is a compound flavonol group of 6 flavonoid subclasses. Flavonoids are a group of compounds in plants that have the same molecular structure of flavones. Quercetin has antioxidant effects, inhibits protein kinase, inhibits DNA topoisomerase and regulate gene expression [9]. 10 mg/kg bodyweight quercetin may inhibit the left ventricular hypertrophy through the increased expression of PPARγ and inhibition of AP-1 signaling pathway. Histopathological test results also showed that the group of spontaneous hypersensitive Rats (SHR) were given therapy quercetin 10 mg / kgBB had the lowest volume of collagen [10]. The C57BL/6J rats are a renal interstitial fibrosis model showed decrease level of TGF-β significantly by giving thiazolidinedione as PPARγ agonist [11].

Administration of quercetin may provide a new discourse use of flavonoid compounds in protecting against renal fibrosis due to excessive salt intake. We need to evaluate the protective effects of quercetin as renoprotective with measured levels collagen volume fraction as a marker for the development of fibrosis in the kidney.

II.METHODS

Five weeks old male Wistar rat (100-150 g) were used in this study. All rats (n=24) were randomized into six groups, and each group consisted of 5 rats. Group 1: negative control, group 2 : rats were induced by intake 8% NaCl doses 2% body weight, group 3: intake 8% NaCl induced rats doses 2% body weight and carboxymethylcellulose 0.5%, group 4: intake 8% NaCl induced rats doses 2% body weight and quercetin 5 mg/kgBB, group 5 : rats were induced by intake 8% NaCl doses 2% body weight and quercetin 10 mg/kgBB, and

group 6: intake 8% NaCl induced rats doses 2% body weight and quercetin 20 mg/kgBB. The 8% NaCl and quercetin or its vehicle (carboxymethylcellulose 0.5%), were given by gavage for eight weeks. After eight weeks rats were killed and the kidney rapidly excised. The left kidney was fixed in 10% formalin solution for collagen volume [1].

For collagen volume fraction analysis, section 5µm thick were deparaffinized, rehydrated and then stained with 0.1% sirrius red in saturated picric acid (Picrosirius red) for 60 minutes. Collagen fraction volume was determined by measuring the area of stained tissue within a field by programme Image J. Within the kidney, fields containing vessels, minor scars were excluded. In kidney 20 fields were analyzed [1].

III. RESULT

Collagen Volume Fraction in Renal Tissue

Group NO (high salt intake) had increased glomerular collagen volume fraction compared with OO (without high salt intake) (p=0.021). Glomerular volume collagen fraction in group NK5 (high salt intake and quercetin 5 mg/kgBB) compared in group NO (only high salt intake) were not significantly different (p=0.773). Group NK10 (high salt intake and quercetin 10 mg/kgBB) compared in group NO (only high salt intake) was not significantly different (p=0.149). Group NK20 (high salt intake and quercetin 20 mg/kgBB) compared in group NO (only high salt intake) were significantly different (p=0.020).

Table 2. Glomerular collagen volume fraction (mean±SEM)

Group	n	Mean±SEM %	*P*
OO	4	3.78±0.12[b]	0.005*
NO	4	19.05±0.99[a]	
NC	4	18.33± 1.39[a]	
NK5	4	19.42± 3.19[a]	
NK10	4	17.14± 0.70[a]	
NK20	4	0.81[b,c,d,e]	

*Kruskal Wallis test

OO: negatif control; NO: rats were induced by intake 8% NaCl doses 2% body weight; NC: rats were induced by intake 8% NaCl doses 2% body weight and carboxymethylcellulose 0.5%; NK5: rats were induced by intake 8% NaCl doses 2% body weight and quercetin 5 mg/kgBB; NK10: rats were induced by intake 8% NaCl doses 2% body weight and quercetin 10 mg/kgBB; NK20: : rats were induced by intake 8% NaCl doses 2% body weight and quercetin 20 mg/kgBB

Post hoc Mann Whitney test :

[a]P<0.05, OO vs NO, OO vs NC, OO vs NK5, OO vs NK10, OO vs NK20

[b]P<0.05, NO vs NC, NO vs NK5, NO vs NK10, NO vs NK20

[c] P<0.05, NC vs NK5, NC vs NK10, NC vs NK20 [d] P<0.05 NK5 vs NK10, NK5 vs NK20

[e]P<0.05 NK10 vs NK20

High salt increased tubular collagen volume fraction (table 3). Group NO (high salt intake) had increased tubular collagen volume fraction compared with OO (without high salt intake) (p=0.021). Tubular collagen volume fraction in group NK5 (high salt intake and quercetin 5 mg/kgBB) compared in group NO (only high salt intake) were not significantly different (p=0.773). Group NK10 (high salt intake and quercetin 10 mg/kgBB) compared in group NO (only high salt intake) was significantly different (p=0.021). Group NK20 (high salt intake and quercetin 20 mg/kgBB) compared in group NO (only high salt intake) there were also significantly different (p=0.021).

Table 3. Tubular collagen volume fraction (mean±SEM)

Group	n	Mean+SEM %	*P*
OO	4	2.26±0.08[b]	0.02*
NO	4	9.79± 0.53[a]	
NC	4	10.19 ±0.24[a]	
NK5	4	9.72± 0.98[a]	
NK10	4	0.49[a,b,c,d]	
NK20	4	1.92± 0.20[b,c,d,e]	

*P<0.05, Kruskal Wallis test

OO: negatif control; NO: rats were induced by intake 8% NaCl doses 2% body weight; NC: rats were induced by intake 8% NaCl doses 2% body weight and carboxymethylcellulose 0.5%; NK5: rats were induced by intake 8% NaCl doses 2% body weight and quercetin 5 mg/kgBB; NK10: rats were induced by intake 8% NaCl doses 2% body weight and quercetin 10 mg/kgBB; NK20: rats were induced by intake 8% NaCl doses 2% body weight and quercetin 20 mg/kgBB

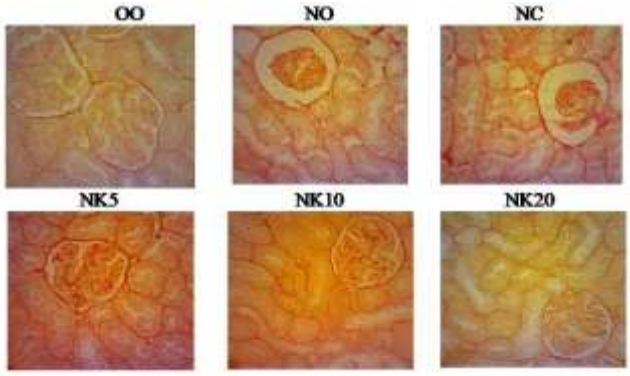
Post hoc Mann Whitney test :

[a]P<0.05, OO vs NO, OO vs NC, OO vs NK5, OO vs NK10, OO vs NK20

[b]P<0.05, NO vs NC, NO vs NK5, NO vs NK10, NO vs NK20

[c] P<0.05, NC vs NK5, NC vs NK10, NC vs NK20 [d] P<0.05 NK5 vs NK10, NK5 vs NK20

[e]P<0.05 NK10 vs NK20



Picture 1. Picrosrius red staining on the renal cortical section under light microscopy (magnification 400x). OO: negative control; NO: rats were induced by intake 8% NaCl doses 2% body weight; NC: intake 8% NaCl induced rats doses 2% body weight and carboxymethylcellulose 0.5%; NK5: intake 8% NaCl induced rats doses 2% body weight and quercetin 5 mg/kgBB; NK10: rats

were induced by intake 8% NaCl doses 2% body weight and quercetin 10 mg/kgBB; NK20: rats were induced by intake 8% NaCl doses 2% body weight and quercetin 20 mg/kgBB

IV. DISCUSSION

The result of this study has important implications that high salt intake was confirmed to rise glomerular and tubular collagen volume fraction to cause renal fibrosis. Some studies show the TGF-β1 mRNA concentration, glomerular and tubular collagen volume fraction in rats fed an 8% NaCl had significantly higher than rats fed a NaCl 1% ($p<0.01$) [7].

Quercetin is the PPARγ ligand that exerts its effect by binding to Ligan Binding Domain (LBD) in the peroxisome proliferator-activated receptor-γ and activating the receptor [12]. PPARγ expressed in many organs including the kidney [13]. Some study support that PPARγ could counteract the profibrogenic effect of TGF-β1 in renal interstitial fibrosis by downregulating the phosphorylation of Smad 2 and Smad 3 [14]. In the present study, we confirmed the protective effect of quercetin in renal fibrosis in Wistar rat induced 8% NaCl. Quercetin 20 mg/kgBB was more lower TGF-β1 than quercetin 5 mg/kgBB and quercetin 10 mg/kgBB but were not significant different. In glomerular volume collagen fraction quercetin were significant different and quercetin 20 mg/kgBB were more lower than quercetin 5 mg/kgBB and quercetin 10 mg/kgBB. In tubular volume collagen fraction quercetin were significant different and quercetin 20 mg/kgBB were more lower than quercetin 5 mg/kgBB and quercetin 10 mg/kgBB.

Some studies support the role of quercetin to protect kidney; quercetin has a protective effect on diabetic glomerulosclerosis in nephropathy by lowering the production of collagen [15]. On the other study quercetin 100 mg/kg for 20 days can reduce the accumulation of collagen in pulmonary fibrosis [16]. In normally, regulation of collagen formation was tightly regulated because excessive collagen accumulation will lead to fibrosis. Most of the cells in kidney such as fibroblasts, tubular epithelial cells, persist, endothelial cells, vascular smooth muscle cells, mesangial cells, and podosit has a role in developing fibrosis [17]. The volume collagen fraction in cardiomyocyte SHR rat with 10 mg/kgBB quercetin lower than the SHR rat with CMC 1% dan 5 mg/kgBB quercetin [10]. PPARγ ligand inhibited collagen transcription by inhibited TGF β1 production and inhibition the formation of the phospo Smad2-Smad3 complex to recruit p-300.

Excessive production of collagen is the renal fibrosis main contributor regulated by TGF-β1 as a fibrogenic factor[19]. The fibrogenic activity of TGF-β stimulated collagen production was demonstrated by TGF-β injection in the neonatal rats[20]. Increase the level of TGF-β1 induced collagen mRNA expression[21]. Rat mesangial cell culture showed that TGF-β level is increasing linearly with increasing mRNAs protein matrix such as biglycan, fibronectin dan collagen. TGF-β antibody inhibits collagen production [22]. Myofibroblast progenitor activated by TGF-β1 and increase collagen production regulation [23]. Research on proximal tubule culture showed TGF-β1 and CTGF induce fibronectin and type IV collagen at the proximal tubule and fibroblast in the cortical kidney area[24]. TGF-β1 induces type IV collagen mRNA and fibronectin expression in human mesangial cells[25]. Collagen and CTGF expression mediated by TGF-β1 has been found in the glomerular mesangial cell, epithelial cell and parietal [26]. TGF-β1 induces collagen expression on NIH-3T3 cell which is *fibroblast-like line derived* from neonatal rat and NRK-49F, the study demonstrated extraglomerular matrix production has an important role in the developing of kidney disease [27].

Some studies indicate TGF-β pathology in renal disease involves signaling through activated receptor serine/threonine kinase (RS/TK) will induce Smad 2/3 (R Smad) phosphorylation and recruit transcription factor to promote gene transcription resulting in cells proliferation, cells, and extracellular matrix (ECM) production. This mechanism promotes kidney fibrosis. Renal fibrosis progresses in some regions of the kidney more quickly than others. TGF-β1 stimulates some cells such as mesangial cells and fibroblast to proliferate and increase the deposition of ECM proteins. Others cells such as tubular epithelial cells, endothelial cells, and podocytes undergo apoptosis. While other epithelial cells dedifferentiate and produce ECM protein, they lost the capability to produce adhesion molecules such as E-cadherin and increase other ECM such as collagen. They undergo cytoskeletal remodeling, rupture tubular basal membrane and the differentiated epithelial cell migrate to the interstitial. Cell structure change cause organ dysfunction [28]. Renal fibrosis promotes CKD and then ESRD, when it is happening, they become *irreversible* renal dysfunction and need dialysis and organ transplantation to life[29].

V. CONCLUSION

Our data indicate that quercetin is suppressing the collage volume fraction with a significant difference in the dose of 20 mg/kg BB quercetin.

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