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Caveolin-1 gene expression in rats model of chronic renal failure

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Abstract

In this study, gene expression profile of caveoline and the kidney MDA and BUN and creatinine levels were investigated in experimentally renal failure case of rats. In the experimental group, rats were injected with 30 mg/kg of cyclosporin A via subcutaneous route for 28 days. In the control group, rats were injected with cremophor EL, vehicle for cyclosporin A, for 28 days. Caveolin gene analysis and MDA analysis in the kidney tissue as well as serum BUN and creatinine analysis were performed at the end of the experiment. Caveolin gene expression of experimental group was significantly reduced ($P < 0.05$), while the MDA level was significantly increased compared to those of control ($P < 0.05$). Serum BUN and creatinine levels were significantly increased in the experimental group compared to the control group ($P < 0.05$). In the Cyclosporin A induced chronic renal failure model, we suggest that the induction of the Cav-1 gene expression may prevent the renal tissue damage.

Keywords: Chronic renal failure, caveolin, cyclosporine A, MDA

Introduction

Chronic renal failure (CRF) can be detected by a low estimated glomerular filtration rate, albuminuria, or both and it is defined by an irreversible, progressive kidney function disorder, observed with biochemical symptoms such as azotemia, hyperphosphatemia, hypocalcemia and generally in a period of longer than three months [1-4]. Caveolae are flask-shaped, 50-100 nm tall invaginations of the cellular plasma membrane, providing mediation of cellular transport such as cholesterol transport, clathrin independent endocytosis; regulation of enzyme functions and receptor signalization [5-7]. Caveolins (Cav) are the main structural proteins that form the caveola [8]. Caveolins have 3 types; caveolin-1, 2 and 3 and Cav-1 expression is especially defined in distal tubule of human kidney and parietal endothelium cells [9]. Cav-1 serves in significant cellular activities such as macromolecular transport, endocytosis of pathogens, lipid metabolism and cell signaling [10]. Concurrently, it has been determined that Cav-1 expression is also related with capillary tubule formation [11]. Taugner et al. [12] reported that renin granules in juxtaglomerular (JG) cells contain caveolin. This study aims to evaluate the change in caveolin gene expression level upon experimental chronic renal failure model in rats.

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Material and Methods

Procurement and care of the rats

An ethics committee approval was obtained from İnönü University, Faculty of Medicine Experimental Animal Local Ethics Committee, dated 09/06/2011 and with protocol no 2011/A-54. 8-10 weeks old female Wistar albino rats were kept in a room with 21º C, 55-60% humidity with 12 hours cycles of dark and light. Rats were fed *ad libitum* with low sodium (0.04%) diet.

Experimental Procedure

Chronic renal failure model was created based on the method described by Andoh et al. [13] with minor modifications. Total of 19 rats were divided into two groups; control group (C; n=10) and chronic renal failure group (CRF; n=9).

Rats in both control group and CRF group were fed with low-sodium diet (0.04% Na) for 5 weeks. Control group rats were subcutaneously given 1 ml cremophor EL solution daily, which is the vehicle solution for cyclosporine A (2 ml cremophor EL solution was mixed with 1 ml 33% ethanol and this mixture was diluted with 0.9% NaCl in 1:5 ratio) and CRF groups rats received 30 mg of cyclosporine A (Novartis, Sandimmun IV ampule; it was prepared by adding 50 mg cyclosporine A containing 1ml ampule in 0.9% NaCl in 1:5 ratio) per kg body weight. Low-sodium diet was used for enhancement of chronic cyclosporine nephrotoxicity. [14]. At the end of the experimental procedure, kidney caveolin-1 mRNA expression and MDA levels were analyzed.

RT-qPCR

Total RNA isolation was carried out from kidney samples using RNeasy Mini Kit (QIAGEN, Hilden, Germany). SuperScript® III Reverse Transcriptase Kit (Invitrogen, Carlsbad, CA) was used for reverse transcription (RT) reactions and the manufacturer's suggested protocol was applied. Caveolin-1 and β-Actin housekeeping gene primer sequences were ordered as obtained from Yang et al. [15] (Table 1). Briefly, PCR amplification mixture (20 µl) contained cDNA, 1 µl of forward primer (10 pmol/ul), 1 µl of reverse primer (10 pmol/ul) and 10 µl of 2x SYBR Green I Master Mix (LC480 SYBR Green I Master Mix,

Roche 04707516001). PCR amplification was performed by the following PCR conditions: 95°C for 10 min; 45 cycles of 95°C for 20 s, 60°C for 20 s, and final heating of 70°C for 30 s. In order to determine the change in Caveolin-1 gene expression in CRF group, β-Actin gene was selected as housekeeping gene and relative mRNA expression levels were calculated according to housekeeping genes using the $2^{-\Delta\Delta Ct}$ method [16]. PCR products were also run in DNA agarose gels and correct sized PCR products were obtained as 159 bp and 304 bp for β-Actin and Caveolin-1 genes, respectively (Figure 1, 2)

Table 1. Primer sequences and the product size for Caveolin-1 and β-Actin. [a]Primer sequences were taken from Yang et al [15]**.**

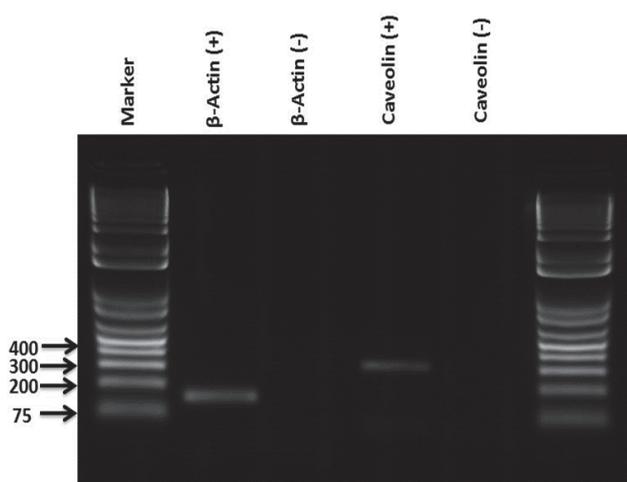| Genes	Primer sequences[a]	PCR product size (bp)
Caveolin-1-F	5'-TCTACAAGCCCAACAACAAGG-3'	304
Caveolin-1-R	5'-AGGAAAGAGAGGATGGCAAAG-3'	
β-Actin-F	5'-CATCACTATCGGCAATGAGC-3'	159
β-Actin-R	5'-GACAGCACTGTGTTGGCATA-3'	

Figure 1. The PCR product size of β-Actin gene and Caveolin gene. After total mRNA extraction of rat kidney, PCR was performed with β-Actin and Caveolin primers (Table 1) and PCR products were run in an agarose gel. Gene Ruler 1 kb plus DNA ladder was used as DNA marker.
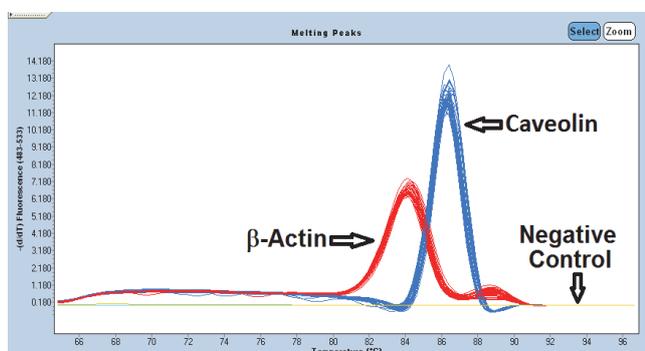

Figure 2. Melting Analysis of Real Time-PCR products of β-Actin and Caveolin mRNA. DNA melting analysis was performed at the end of the PCR.

Tissue MDA levels

The malondialdehyde (MDA) levels of kidney homogenates were assayed spectrophotometrically at 535 and 520 nm according to Mihara and Uchiyama [17]. The results were expressed as nanomoles per gram of wet tissue (g.w.t).

Blood urea (BUN) and creatinine measurements

BUN and creatinine levels were determined using an auto analyzer (Abbott Laboratories. Abbott Park, Illinois, U.S.A.). Body weight, BUN and creatinine levels were measured at the beginning and at the end of the experimental period for both control and CRF groups.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences software 19.0 for Windows package software (SPSS, Inc., Chicago, IL). The normality of distributions of data was evaluated with the Shapiro-Wilk test and gene expression levels was found normal (P>0.05). The statistical comparisons of gene expressions and kidney MDA values were implemented using independent sample t-test. The statistical comparisons of pretest and posttest weight, BUN and creatinine values of animals were implemented using paired t-test. Data were given as mean ± standard deviation. Values with less than 0.05 p value were determined as statistically significant.

Results

The findings for body weight changes for both control and CRF groups, BUN and creatinine levels were show in Table 2.At the beginning of the experiment, it was found that, body weight, BUN and creatinine levels were similar in both groups (p>0.05). At the end of experimental procedure, the average weight of control group animals did not change, however, CRF group animals lost weight (p<0.05). BUN and creatinine levels of experimental CRF

animals were higher (p<0.05), although, those levels did not alter in control group animals (Table 2).

MDA and Caveolin/β-Actin mRNA level ratios were also measured at the end of the experiment for C and CRF

groups. It was observed that MDA level was significantly higher than in CRF group (p<0.05). mRNA level of Caveolin decreased in CRF group when compared to the C group (p<0.05) (Table 3).

Table 2. Body weight, BUN and creatinine levels. Body weight, BUN and creatinine levels were measured in both control (C) group and chronic renal failure (CRF) group animals at day 0 and day 35. Letters different from each other in columns depict statistically significant difference between each other (p<0.05).

	Body Weights (gr)		BUN (mg/dl)		Creatinine (mg/dl)	
Days	C (n=10)	CRF (n=9)	C (n=10)	CRF (n=9)	C (n=10)	CRF (n=9)
Day 0	237.9 ± 15.9^a	249.8 ± 33.5^a	19.2 ± 2.7^a	17.6 ± 1.6^a	0.48 ± 0.03^a	0.47 ± 0.03^a
Day 35	231.2 ± 17.9^a	210.2 ± 25.2^b	17.5 ± 1.6^a	32.2 ± 6.7^b	0.50 ± 0.06^a	0.59 ± 0.07^b

Table 3. MDA level and Caveolin/β-Actin mRNA level ratios. MDA level and Caveolin/β-Actin mRNA level ratios were measured at the end of the experiment. Letters different from each other in columns depict statistically significant difference between each other (p<0.05).

Groups	MDA (nmol/gwt)	mRNA level of Caveolin/β-Actin
C (n=10)	54.5 ± 10.1^a	0.0050 ± 0.0008^a
CRF (n=9)	165 ± 8^b	0.0039 ± 0.0006^b

Discussion

The study aimed to examine the result of chronic renal failure in kidney by evaluating biochemical and molecular parameters. The renal Cav-1 gene expression, renal MDA levels and serum creatinine and BUN levels in the rat chronic renal failure model was measured after creating CRF case. At the end of the experimental period, renal Cav-1 gene expression level of CRF group were found to be statistically lower as compared to control group animals ($p < 0,05$) whereas renal MDA levels of CRF group were statistically higher than control group ($p < 0,05$). In addition, serum creatinine and BUN levels were statistically higher in the CRF group ($p < 0,05$).

Several studies have been conducted examining the changes in Cav-1 expression in renal failure. Bai et al. [18] have examined the effects of neurogenic hypertension-induced renal damage model on Cav-1 protein expression in rat kidney. They have established the hypertension model by injecting single dose phenol and detected a prominent decrease in Cav-1 protein expression in hypertensive kidney.

Three independent pathways may regulate the decrease in Cav-1 gene expression at the transcriptional level; Ras-p44/42 MAPK, non-receptor tyrosine kinases and protein kinase A [19]. Cav-1 expression can be inhibited through transcriptional activation of a mitogen sensitive promoter and mediated by Ras/MAPK, or it can be directly regulated by the activation of a cascade of p44/42 MAP kinase [19]. The decrease in Cav-1 gene expression, in our study, might be originated from increasing intracellular Ras-p44/42 MAPK activity or PKA activity [19].

Another reason for the decrease in Cav-1 expression might be due to the reduction in intracellular calcium by Cyclosporin A. Yang et al. [20] stated that they investigated calcium regulation of Cav-1 gene expression at transcriptional level at metastatic rat osteosarcoma cell lines inducted by FBJ-S1 virus with high amounts of Cav-1 gene expression from BALB/c rats. When FBJ-S1 cells were treated with Cyclosporin A, caveolin-1 gene expression and Cav-1 protein levels in these cells have decreased. This decrease could have been due to the increased intracellular calcium concentration resulting in the activation of the calmoduline, which causes the activation of calcineurin that is blocked by Cyclosporine A. The same authors concluded that, Cav-1 gene expression could be regulated positively at the transcriptional level with a new calcium signaling pathway mediated by L-type calcium canal/Ca^{+2} / calcineurin / NFAT.

The decrease in the renal Cav-1 gene expression was also attributed to the oxidative stress by Fakhrzadeh et al. [21]. The authors notified that Cav-1 expression decreased in alveolar macrophages of mice that were exposed to a powerful oxidant and ozone by inhalation. The authors showed that TNF-α stimulated NO production, especially in macrophages and contributed to the pathogenesis of ozone mediated oxidative tissue damage and then inhibited Cav-1 expression. The decrease in CAV-1 expression might occur because of the conformational alteration due to the phosphorylation of Cav-1 by TNF-α via p44/42 MAP kinase pathway [21].

We determined that, MDA levels, one of the identifiers of free radical induced damage in tissue increased in the chronic renal failure group. Several studies have stated the increase in lipid peroxidation is the result of chronic renal failure, which supports our findings of this study. Increasing MDA levels could be explained by Cyclosporin A induced free oxygen radicals [22].

Sener et al. [23] described increased tissue MDA levels in the chronic renal failure, whereas GSH values was shown to be decreased. Also, Rezzani [24] reported that Cyclosporin A caused increased lipid peroxidation

products and tubular fibrosis in kidney tissue. Similarly, it has been demonstrated that Cyclosporin A application increased MDA level in kidney cortex of rats [25].

The reason for the chronic renal failure upon Cyclosporin A administration might have decreased Cav-1 gene expression which might have induced intracellular calcium decrease, might have activated p44/42 MAPK or PKA levels. This activation might increase oxidative stress which may lead to decreased Cav-1 gene expression levels [19-21].

Cav-1 is a crucial molecule in signaling pathway and maintaining the integrity of caveolae [26]. Decrease in Cav-1 can be regarded as one of the essential mechanisms in the renal damage that leads to the deterioration of the integrity of the caveola. The regulatory molecules (such as src, EGFR, ras, G protein α subunit, adenylate cyclase, protein kinase A, etc.) which are mostly located on the caveola membrane and have roles in signaling pathway may deteriorate when the Cav-1 gene expression decreases [19]. Garcia et al. [27] have asserted that Cav-1 was a powerful biological marker of the renal fibrosis in the neonatal obstructive nephropathy process.

Many studies have suggested using the Cav-1 as a therapeutic target due to its low expression levels in fibrosis. In the Cyclosporin A induced chronic renal failure model, we suggest that the induction of the Cav-1 gene expression may prevent the renal tissue damage.

Conflict of interest: Authors declare that there are no conflicts of interest.

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