Experimed 2023; 13(2): 93-96

ORIGINAL ARTICLE

DOI: 10.26650/experimed.1291728

Cyclosporine Treatment Increases the ACE/ACE2 Ratio in Adipose Tissue and Aorta

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Cite this article as: Darilmaz SE, Bingul I, Soluk-Tekkesin M, Demir N, Bekpinar S. Cyclosporine treatment increases the ACE/ACE2 ratio in adipose tissue and aorta. Experimed 2023; 13(2): 93-96.

ABSTRACT

Objective: The renin-angiotensin system (RAS) mainly functions in the regulation of vascular tone. Since the perfusion and storage capacity of adipose tissue is largely dependent on vessel density and tone, factors that modulate vasoactive RAS components may affect adipose tissue metabolism. Cyclosporine, an immunosuppressive drug, has some detrimental effects on the vascular system and kidneys by modulating the components of the RAS. However, the effect of cyclosporine on adipose tissue is still not clear. In this study, the impact of cyclosporine on mRNA expressions of some "RAS components" in the aorta and epididymal adipose tissue of rats were investigated.

Materials and Methods: Rats were injected subcutaneously with cyclosporine at a dose of 25 milligrams per kilogram per day for 1 week. Angiotensin II, angiotensin (1-7), blood urea nitrogen and creatinine levels were examined in serum samples of rats. mRNA expressions of RAS components in the aorta and epididymal adipose tissue, and hypoxia-inducible factor-1 (HIF-1) and vascular endothelial growth factor (VEGF) in epididymal adipose tissue were determined. In addition, histopathological examinations of the adipose tissue were performed.

Results: Cyclosporine administration significantly suppressed the expression of angiotensin-converting enzyme 2 (ACE2) in both the aorta and adipose tissue. Accordingly, it caused a significant increase in the ACE/ACE2 ratio. However, it did not affect the adipose tissue HIF-1 and VEGF expressions. When examined histopathologically, no change was observed in the vascular density of adipose tissue due to cyclosporine.

Conclusion: It was thought that the increase in the ACE/ACE2 ratio might be an adaptation that protects the adipose tissue against the systemic effect of cyclosporine.

Keywords: Cyclosporine, adipose tissue, angiotensin-converting enzyme 2, vascular density

INTRODUCTION

Cyclosporine is an immunosuppressant medication used in clinical practice to prevent organ graft rejection and to treat autoimmune diseases. However, this drug shows additional effects on non-immune cell populations and may adversely affect the vascular system and kidneys (1).

It has been reported that upregulation of the vasoconstrictive renin-angiotensin system (RAS) and endothelin-1, inhibition of nitric oxide (NO)-dependent vasodilation, and also increasing the formation of free radicals are effective in the hypertensive and nephrotoxic effects of cyclosporine (1).

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Submitted: 03.05.2023 **Revision Requested:** 24.05.2023 **Last Revision Received:** 25.05.2023 **Accepted:** 05.0.2023 **Published Online:** 26.07.2023

Table 1. Serum parameters of control and cyclosporine-treated rats.

	CONTROL (n=7)	CYCLOSPORINE (n=9)
Ang-II (pg/mL)	66 ± 2.7	61 ± 5.0
Ang 1-7 (pg/mL)	59 ± 1.6	48 ± 3.1 [a]

Note: Values are means ± SEM. In statistical evaluations, a Mann–Whitney U test were used.The unpaired Student's t-test was used for equal variances. Ang-II, angiotensin II; Ang (1-7), angiotensin (1-7).
[a]: $p < 0.05$ compared with the control group.

Table 2. Vascular density score in epididymal adipose tissue in control and cyclosporine-treated rats.

	Vascular density score
Control group	10.83 ± 0.74
Cyclosporine-treated group	12.25 ± 0.62

Note: Values are mean± SEM (*n* = 7–9 in each group). Vessels in three different areas of adipose tissue section of each rat were counted and averaged. In statistical evaluations, a Mann–Whitney U test were used.

Angiotensin II (Ang-II), which is synthesized by catalysis of angiotensin-converting enzyme (ACE), exhibits a vasoconstrictive effect when it interacts with the Ang-II type-1 receptor (AT1R), whereas a vasodilator effect is observed by interacting with the Ang-II type-2 receptor (AT2R). Moreover, angiotensin (1-7), synthesized by angiotensin-converting enzyme-2 (ACE2) enzymatic activity, has a vasodilator effect when it binds to the Ang (1-7) receptor (Mas R). The antagonistic effects of RAS components are crucial in the regulation of vascular tone and perfusion of the relevant organ.

RAS components also exert a crucial role in adipose tissue metabolism in an autocrine or paracrine manner (2,3). It has been reported that RAS components with vasoconstrictive effects are largely responsible for hypertension that seen in obese individuals (2, 3).

Although its apparent effects on the vascular system and kidneys are known, how cyclosporine affects adipose tissue metabolism has not been studied much. In a study administering to rats by gavage for six weeks, cyclosporine was reported to decrease glucose uptake into adipocytes, increase lipolysis and suppress lipogenesis (4).

In this study, we aimed to elucidate the effect of cyclosporine administration to rats for 1 week in mRNA expressions of RAS components in both epididymal adipose tissue and the aorta. In addition, we also elucidated the vascular density and the mRNA expressions of HIF-1 and VEGF in adipose tissue.

MATERIALS AND METHODS

Animals

In this study, sixteen male Wistar rats weighing approximately 350 grams, provided by the Bezmialem University, Experimental Medical Research Institute were housed in a stainless cage (three to four per cage) with temperature and light control (12 h dark/12 h dark) and received a standard pellet diet. The experimental procedures were implemented according to the order of Bezmialem University, Animal Experiments Local Ethics Committee (Date: 28.02.2019, No: 33).

Experimental Design

Cyclosporine group (n=9): Cyclosporine A (Sandimmune, Novartis, Switzerland) was prepared in ethanol (94%) and polyoxyethylated castor oil and a dose of 25 mg/kg was applied subcutaneously once daily during the one-week experimental period, according to previous studies (5).

Control group (n=7): Rats were exposed to daily subcutaneous injections of Cremophore EL: alcohol (2:1) as a vehicle during the one-week experimental period.

Samples

Rats were given ketamine (35 mg/kg, Pfizer, USA) and xylazine HCl (15 mg/kg, Bioveta, Czech Republic) intraperitoneally for anaesthesia, and euthanized after an overnight fasting. Blood samples were taken from their hearts into tubes; thereafter, sera were obtained by centrifugation.

The thoracic aorta and epididymal adipose tissue samples were isolated from each rat.

Determination of Ang-II, Ang (1-7) , Blood Urea Nitrogen (BUN) and Creatinine in Serum

Ang-II and Ang (1-7) (Rat ELISA kit; Ang II #101212; Ang (1-7) #101211; Abbkine, Inc., China), as well as BUN and creatinine (Roche Cobas auto-analyzer) levels in sera were determined.

Determination of mRNA Expressions in the Aorta and Adipose Tissue

AT1R, ACE, and ACE2 mRNA expressions in the aorta and ACE, ACE2, HIF-1 and VEGF mRNA expressions in the epididymal fat sample were measured. A commercial kit (NucleoSpin RNA Isolation Kit, #740955, Macherey-Nagel, Germany) was used for the RNA purification. Following that procedure, 5 ng purified RNA was also used to synthesize the cDNA samples (cDNA Synthesis Kit; #PCR511, Script, Jena-Bioscience GmbH, Jena, Germany). After the cDNA acquisition, quantitative real time-polymerase chain reaction (qRT-PCR) was carried out by using the qPCR Sybr Green Master Mix kit from Jena Bioscience (#PCR372, GmbH, Jena, Germany) with the following primers of AT1R (NM_004835, F:5'-TTCACCCTGCCTCAGGATCT-3',

R:5'-CCAGACCCACCAATCCA TCC-3');

ACE (NM_000789, F:5'-CGTCCACCGTTACCAGACAA-3',
R:5'-TTGGCCTCTGCGTATTCGTT-3');
ACE2 (NM_021804, F:5'-GAATGCGACCATCAAGCGTC-3',
R:5'-CAAGCCCAGAGCCTACGATT-3');
VEGF (NM_001025366, F:5'-GCACTGGACCCTGGTTTAC-3',
R:5'-GGGTCTCAATTGGACGGCAA-3') and
HIF-1 (NM_001530, F:5'-ATGTACCCTAACAAGCCGGG-3',
R:5'-AAGCACGTCATAGGCGGTTT-3') (LGC Biosearch Technologies, Denmark) in a Biorad-CFX Connect device (California-USA). The relative mRNA expressions were calculated with the method of $2^{-\Delta\Delta CT}$. The expression of mRNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an internal control, was used to adjust the analytical data.

Histopathological Examination

Samples of epididymal adipose tissue were preserved in a 10% buffered formalin solution, which was then paraffin blocked. Hematoxylin-eosin stained preparations were observed morphologically under a light microscope. For scoring of the vascular density, three different areas were chosen, and then the number of vessels was counted and vascular density was calculated by dividing the total number of vessels by three.

Statistical Analyses

The statistical analyses were implemented by using the Statistical Package for The Social Sciences (21.0; SPSS Inc., Chicago, IL-USA) program, unpaired Student's ttest or Mann-Whitney Utest, according to data distribution. Correlations were evaluated by Pearson's test. When p-values are less than 0.05, they were considered significant.

RESULTS

Cyclosporine administered daily for one week into animals at a dose of 25 mg/kg, did not affect renal function; BUN and creatinine levels in serum did not change (Cyclosporine: 24.7 ± 1.1 mg/dL - Control: 25.6 ± 4.7 and Cyclosporine: 0.36 ± 0.01mg/dL - Control: 0.31 ± 0.03 respectively). While serum Ang- II levels were not affected by cyclosporine treatment, Ang (1-7) levels decreased (Table 1).

Cyclosporine treatment in aortic tissue significantly increased the mRNA expression of AT1R. However, a significant decrease in ACE2 expression and an increased ACE/ACE2 ratio were observed (Figure 1).

A significant cyclosporine-induced decrease in ACE2 expression was also found in adipose tissue, resulting in a marked increase in the ACE/ACE2 ratio. However, AT1R and ACE expressions remained unchanged with cyclosporine treatment (Figure 2). To evaluate the effect of cyclosporine on adipose tissue perfusion, we examined histopathologically the vascular density and RNA expressions of HIF-1 and VEGF. We did not detect any change in these parameters due to cyclosporine (Figures 2 and 3, Table 2).

But interestingly, a significant negative correlation was found between ACE/ACE2 ratio and HIF-1 in adipose tissue (r= -0.651, p=0.009).

DISCUSSION

The effect of the administration of 25 mg/kg cyclosporine to rats for one week on mRNA expressions of RAS components in the aorta and epididymal adipose tissue were investigated in the current study. In the aorta, this immunosuppressive drug caused an increase in AT1R and a decrease in ACE2 expression and a marked increase in the ACE/ACE2 ratio. In addition,

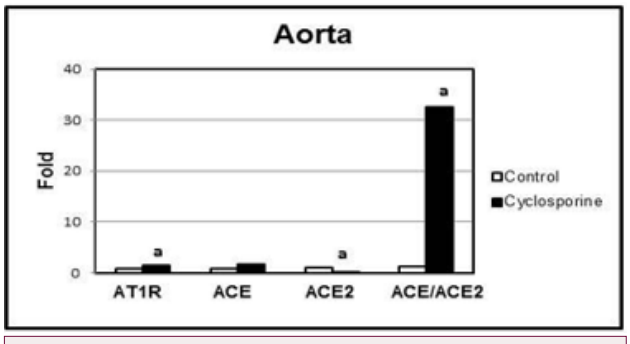

Figure 1. The impact of cyclosporine on mRNA expressions of RAS components in rat aorta

Values are given as mean ± SEM (*n*=7-9 for each group).

a: p < 0.05; compared to the control. AT1R, angiotensin-II type-1 receptor; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme-2

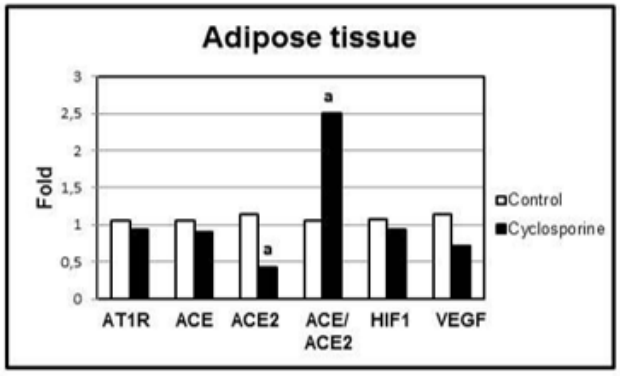

Figure 2. The impact of cyclosporine on mRNA expressions of RAS, HIF-1 and VEGF in rat epididymal adipose tissue

Values are given as mean ± SEM (n=7-9 for each group).

a: p < 0.05; compared to the control. AT1R, angiotensin-II type-1 receptor; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme-2, HIF-1, hypoxia-inducible factor-1; VEGF, vascular endothelial growth factor

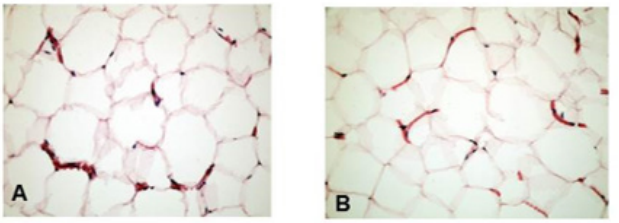

Figure 3. The representative images of the epididymal adipose tissue stained by hematoxylin-eosin (n=7-9 in each group). Control (A) and cyclosporine (B) groups have a similar appearance. Thin vessel sections were observed between adipocytes. (Original magnification×400)

serum Ang-II levels remained unchanged, but Ang (1-7) levels were found to be low in the cyclosporin group. Our findings showed that at least in part that cyclosporine has a systemic vasoconstrictive effect, as previously described (1, 6, 7).

Adipose tissue is a highly vascularized organ. Beyond supplying oxygen and nutrients, the vascular system carries a variety of cytokines, growth factors, and hormones to and from adipose tissue. Any modulation of vessel density and vessel diameter of adipose tissue affects tissue perfusion and function. The main transcription factor regulated by oxygen homeostasis and hypoxia is HIF-1. Under hypoxic conditions, HIF-1 activates the transcription of genes facilitating metabolic adaptation to hypoxia such as glycolytic enzymes, vascular endothelial growth factor, etc. (8)

In our study investigating the effect on some vasoactive RAS components and hypoxic parameters in adipose tissue, it was found that cyclosporine decreased ACE2 expression and increased the ACE/ACE2 ratio. However, HIF-1 and its target gene VEGF expressions were not affected by this drug. Furthermore, no change was observed histopathologically in the vascular density of adipose tissue due to cyclosporine. But interestingly, a marked inverse association was detected between the ACE/ACE2 ratio and HIF-1 in adipose tissue.

It is well known that the increase in systemic blood pressure passively dilates the vessels in the resistant arteries and arterioles embedded within the parenchyma. To compensate for this expansion, the smooth muscle cells of the vessel contract and try to return the vessel diameter to its initial state. This reactive contraction, called the myogenic response, counteracts the effect of a change in systemic pressure on tissue perfusion (9, 10). Multiple mechanisms have been reported to mediate the myogenic response depending on their location in the body (9). In a study conducted in cerebral resistant arteries, the role of AT1R activation in the pressure-induced myogenic response was indicated (11).

According to our findings, the increase in ACE/ACE2 ratio observed due to cyclosporine in our study may be related to the myogenic adaptation that protects the adipose tissue against the systemic effect of cyclosporine.

Ethics Committee Approval: This study is approved by Bezmialem University, Animal Experiments Local Ethics Committee (Date: 28.02.2019, No: 33).

Authors' Contributions: Conception/Design of Study – S.B, I.B.; Data Acquisition – S.B., I.B.; Data Analysis/Interpretation – S.B., I.B., E.D.; Drafting Manuscript– I.B.; Critical Revision of Manuscript- S.B.; Final Approval and Accountability– I.B.; Technical or Material Support- E.D., I.B.; M.S.T, N.D.; Supervision- S.B.

Conflicts of Interest: The authors declare no conflict of interest.

Financial Disclosure: The authors declare that this study has received no financial support.

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