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Research Article

Effects of Cyclosporin A, Mycophenolate Mofetile, Vitamin A, D, E and N-Acetyl Systein in Rats With Nephrotic Syndrome

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Abstract

Objectives: Patients with steroid-resistant nephrotic syndrome have a high risk of developing chronic renal failure. Cyclosporine A used in treatment is nephrotoxic. Data on the efficacy of mycophenolate mofetil used in the treatment are insufficient. New treatment options should be explored. Our research aims to investigate the effectiveness of cyclosporine A, mycophenolate mofetil, vitamin A, D, E, N-acetyl cysteine and their combinations in rats with nephrotic syndrome.

Methods: The research was conducted with 48 adult male rats of the Wistar-albino. To induce nephrotic syndrome, two doses of adriamycin were administered to all rats 20 days apart. Serum creatinine, albumin, cholesterol, triglyceride, total oxidant status, total antioxidant status, protein in urine, creatinine, and creatinine clearance were analyzed in rats. Glomerulosclerosis index, total injury score, interstitial fibrosis score, TGF-and osteopontin analyzes were performed within the scope of the histopathological evaluation.

Results: At the end of the study, the lowest 24-hour urine protein was found in group B (cyclosporin A + vit ACE), and the highest 24-hour urine protein in group C (mycophenolate mofetil). The highest serum creatinine, triglyceride, and cholesterol and the lowest serum albumin levels are in group A (cyclosporine A). The lowest serum creatine, triglyceride, and cholesterol and the highest serum albumin levels are in group D (mycophenolate mofetil + vit ADE).

Conclusion: It has been observed that vitamin ACE added to cyclosporine A treatment reduces cyclosporine A nephrotoxicity, and vitamin ACE added to mycophenolate mofetil contributes positively to renal histopathological findings.

Keywords: Nephrotic syndrome, Cyclosporine A, Mycophenolate mofetil, Vitamins A, D, E, N-Acetyl cysteine.

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Nephrotic syndrome (NS) is a disease characterized by intense proteinuria, hypoalbuminemia, hyperlipidemia, and edema findings resulting from impaired protein permeability of glomeruli. Focal segmental glomerulosclerosis (FSGS), renal histology that is more common in patients with steroid-resistant nephrotic syndrome (SDNS), is a poor prognostic sign. The probability of response to the immunosuppressives used is low, and side effects are common.[1]

Today, the use of complementary therapies is accepted in cases where there is no response to immunosuppressives. There is a need for new options that are effective and have no side effects in the treatment of NS. New immunosuppressives or new complementary therapies should be researched.[2]

By using adriamycin on rats, an NS model with FSGS histology can be created. Adriamycin-induced NS pathogenesis includes apoptosis. Damage develops in the visceral epithelial cells of the glomeruli, resulting in severe proteinuria, glomerular sclerosis, and diffuse tubulointerstitial damage.[3,4]

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Widely used in transplantation patients, cyclosporine A (CsA) has an antiproteinuric activity and is used in the SDNS treatment. However, vasoconstriction and tubulointerstitial fibrosis in the kidneys limit its use.[5,6]

Mycophenolate mofetil (MMF), the most commonly used immunosuppressive with CsA, has an antiproteinuric property. Recently, it has been tested in the treatment of steroid-dependent NS. It shows its antiproteinuric effect through vascular cellular adhesion molecule-1 (VCAM-1). MMF has no known nephrotoxic properties to date and is accepted as a renoprotective agent. Animal experiments in MMF and NS models are few and comparative studies with other immunosuppressives are required.[7]

Antioxidants can be considered as an option to increase the effectiveness of immunosuppressives and reduce their side effects. The antioxidant, immunomodulatory and antifibrotic properties of vitamins A, D, and E (vit ADE) and N-acetylcysteine (NAC) have been the subject of various studies, but the effects of their combined use have not been adequately studied.[8]

In SDNS patients, oxidative stress occurs in the kidney tissue with the effect of nephrotoxic drugs such as CsA. Correspondingly, free oxygen radicals concentrate at the tissue level and trigger a series of immune reactions. As a result, fibrosis developing in the kidney tissue progresses to sclerosis and end-stage renal disease (ESRD) develops.[9]

Due to its antioxidant and antifibrotic properties, vit ADE may be an effective complementary treatment option for NAC, SDNS. It is evaluated that the efficacy of treatment can be increased and the side effects of immunosuppressives can be reduced by adding a combination of vit ACE to immunosuppressives in NS-induced rats. In addition, vit ACE and NAC may be effective in the treatment of NS-induced rats in cases where immunosuppressive therapy cannot be given.

Our aim is to examine the efficacy and side effects of vitamin vit ADE, CsA, MMF, and NAC alone or in combination in rats with NS. For this purpose, laboratory markers of NS, renal function tests, and renal histopathological findings were analyzed.

Methods

48 adult male rats of the Wistar-albino breed, weighing 200-300 g, were included in the study. Six groups were formed, with eight rats in each group. On the first day, all rats were placed in metabolic cages and their urine was collected, and the next day, blood samples were taken from their tail veins under light anesthesia. These urine and blood samples used were used as controls. Adriamycin (Adriblastina; Pharmacia Upjohn Milano, Italy) was administered intravenously at a dose of 2 mg/kg to all rats in the tail vein on days 1 and 21 3. Working groups are organized as follows:

Group A: CsA

Group B: CsA+Vit ADE

Group C: MMF

Group D: MMF+Vit ADE

Group E: Vit ADE

Group F: NAC +Vit ADE.

All treatments were started on the 22nd day after the second dose of adriamycin. CsA 25 mg/kg/day (Sandimmun Neoral; Sandoz Ltd. Basel, Switzerland) via nasogastric tube (NGS), MMF (Cellcept; Roche Pharmaceuticals Basel, Switzerland) 20 mg/kg/day with NGS, vit A 10 mg/kg /day (Roaccutane; Roche Pharmaceutical Basel, Switzerland) with NGS, vit D3 0.5 mcg/kg/ga (Calcijex; Abbott Laboratories North Chicago, USA) intraperitoneally, vit E 600 mg/kg/day (Evigen; Aksu Farma Medical Products Pharmaceuticals) Sanayi AŞ Istanbul, Turkey) subcutaneously, NAC 40 mg/kg/day (Assist; Hüsnü Arsan İlaçları AŞ Istanbul, Turkey) intraperitoneally. All drugs given by nasogastric tube are given in 0.5 mL olive oil to facilitate absorption and to give in the same volume. Doses and routes of administration of drugs were taken from reference studies in the literature.[10-14]

The rats were fed with normal rat chow and tap water throughout the experiment, no water and feed restrictions were applied. Their weights were measured twice a week and their systolic blood pressure once a month. The signals received with the pressure probe attached to the tail were transferred to the computer via the MP 100A-CE data acquisition system (BIOPAC Systems, CA-USA) and the MAY-BPHR200 unit, and the measurements were made with the pressure traces drawn with the "Acknowledge" package program. Measurements were continued until the end of the experiment. Three successful measurements were averaged each time.

The duration of treatment was determined as 16 weeks. Blood and 24-hour urine samples of the rats were collected once before the treatment and five times after the treatment, at the 4th, 8th, 12th, and 16th weeks. All blood and urine samples were stored at -70 °C until a biochemical study was performed.

Serum and urine creatinine levels were measured using the Roche rate-blanked and compensated Jaffe method using original Roche kits. Simultaneous 24-hour urine volumes with serum and urine creatinine and creatinine clearances according to the body surface of rats were calculated as ml/min/1.73m^2. Serum total protein and albumin levels were measured spectrophotometrically using original Roche kits. Serum total cholesterol and triglyceride levels were measured by the enzymatic spectrophotometric method using original Roche kits. Urine total protein levels were

measured using the turbidimetric method, using original Roche kits. All measurements were made on a modular PPP autoanalyzer (Roche Diagnostics, GmbH, Mannheim), results are given in mg/dL or g/dL.

Serum total oxidant status (TOS) and serum total antioxidant status (TAS) analyzes were performed spectrophotometrically on a V-Twin autoanalyzer (Dade Behring, Syva, Marburg, Germany) using the Erel method. The results are given in μmol H_2O_2 Equiv./L and μmol Trolox equivalent/L, respectively.[15,16]

Since studies in the literature reported that glomerulosclerosis and tubulointerstitial damage occurred at the 16th week in NS models created with adriamycin, the rats were sacrificed at the end of the 16th week. The kidneys were removed and weighed, fixed with 6% neutral formalin, and then covered with paraffin. Sections with a thickness of 3 mm were stained with hematoxylin-eosin, Masson's trichrome, TGF-β, and osteopontin (OPN) and evaluated by pathologists.

To determine the degree of glomerular sclerosis, the semiquantitative scoring method of Raij et al. was used. With this method, the glomerular sclerosis index (GSI) and the total injury score (THS) for each preparation were calculated. GSI was calculated by taking the average of the total score of 20 glomeruli for each rat kidney section in hematoxylin-eosin-stained preparations. If the lesion covers less than 25% of the glomeruli, it is scored as 1(+), if 25-50% is scored as 2(+), if 50-75% is scored as 3(+), if 75-100% is scored as 4(+). For example: If 12 out of 20 glomeruli have 1(+), 1 have 2(+), 1 have 3(+) lesions and 6 glomeruli have no damage, it is calculated as 0.85 by dividing the total score of 17 by 20. The injury score was determined by multiplying the degree of injury [from 0 to 4(+)] by the percentage of glomeruli with the same degree of injury. THS for each tissue sample was obtained by summing these scores. As an example: in a tissue sample, if 9 out of 20 glomeruli have 1(+), one has 2(+) lesions and 10 glomeruli have no lesions, THS for this example is [(1x9/20)+(2x1/20)+(0x10/20))] x 100 = 55.[17]

In preparations stained with Masson's trichrome for interstitial fibrosis scoring (IFS), fibrotic areas in the cortical and corticomedullary region over the tubulointerstitial area were counted. Spots taking dye in the medullary region were not evaluated. The degree of interstitial fibrosis was determined by the standard point counting method using the ocular grid. A 21x21 ocular grid containing 441 points was placed on the ocular part of the light microscope, and the points falling on the painted area were counted with Masson's trichrome at 40 magnifications. The arithmetic means of the results of 10 consecutive regions that do not intersect in the interstitial area in the biopsy samples was calculated as IFS.

Interstitium for TGF-β staining and tubules for OPN staining were evaluated. The areas stained with TGF-β and OPN on the tubulointerstitial area in the cortical and corticomedullary region were counted using the standard point counting method using the ocular grid. A 21x21 ocular grid containing 441 points was placed on the ocular part of the light microscope, and the points falling on the stained area with TGF-β and OPN at 40 magnifications were counted. The arithmetic means of the results of 10 consecutive regions that do not intersect in the tubulointerstitial area in the biopsy samples was calculated as TGF-β and OPN scores.

The data of each group were compared within and between groups, and which treatment combination was more effective was investigated. Mann Whitney U test was used for statistical comparison of data between groups. Data evaluations within the groups according to the weeks were made by analysis of variance in repeated measurements, and the differences were compared with the Bonferroni correction. Mean±standard deviation (±SD) was given as descriptive statistics, and $p<0.05$ was accepted as significant.

Results

The drugs given in our study (CsA, MMF, Vit ADE, NAC) were well tolerated by the subjects. During the study, a total of six rats, two from group A, three from group B, and one from group F died and were not included in the analysis.

1. Body Weight Tracking of Rats

The increase in body weights in group A (CsA) and group B (CsA+vit ADE) rats was found to be the lowest in all groups at the end of the 16th week. There was a significant increase in body weights between the 4th and 8th weeks in group A (CsA), group D (MMF + Vit ADE), and group E (vit ADE) rats, and a significant decrease in group B (CsA + vit ADE) rats. In Group C (MMF) rats, a significant increase in body weight was detected between the 8th and 12th weeks.

2. Kidney Weights

Mean kidney weights were determined as group A: 1.18±0.11 g, group B: 1.48±0.33 g, group C: 1.20±0.17 g, group D: 1.35±0.22 g, group E: 1.23±0.08 g, group F: 1.31±0.10 g. There was no significant difference between the groups in terms of kidney weights.

3. Systolic Blood Pressure Values

The mean systolic blood pressures of the groups were similar at 0, 4, 8, and 12 weeks. In Group E (vit ADE) rats, mean systolic blood pressures at week 16 were found to be significantly higher than their measurements at weeks 8 and 12. Again, mean systolic blood pressures of group E (vit ADE) at week 16 were found to be significantly higher than group A (CsA) and group C (MMF).

4. 24-Hour Urine Volumes

Urine volumes of group A (CsA) and group B (CsA + vit ADE) rats were significantly reduced compared to other groups, especially at 16 weeks. Urine volumes of group C (MMF) and group D (MMF+vit ADE) rats were found to be higher at 16 weeks compared to other groups.

5. Proteinuria Levels

In Group A (CsA) rats, 24-hour urine protein levels at week 4 were found to be significantly lower than all other groups but increased significantly at week 12. Group C (MMF) rats at week 16 and Group D (MMF+vit ADE) at week 12 had significantly higher 24-hour urine protein levels.

The lowest 24-hour urine protein values were in group A (CsA) at week 4, group D (MMF+vit ADE) at week 8, group E (vit ADE) at week 12, and group E (CsA+) at week 16. vit ADE) was measured.

6. Serum Albumin Levels

Among the groups, serum albumin levels of group B (CsA+vit ADE) were found to be significantly lower than group E (Vit ADE) only at week 16. Although not statistically significant, serum albumin levels of group D (MMF+vit ADE) rats at week 16 were significantly higher than group A (CsA) and group B (CsA+vit ADE) rats.

7. Serum Cholesterol Levels

Serum cholesterol levels of Group D (MMF+vit ADE) at 8, 12, and 16 weeks were lower than all other groups. Of these, group A (CsA) at weeks 8, 12, and 16; group B (CsA + vit ADE) at weeks 12, and 16 are significant.

8. Serum Triglyceride Levels

Serum cholesterol levels of Group D (MMF+vit ADE) at 8, 12, and 16 weeks were lower than all other groups. Serum triglyceride levels of group A (CsA) and group B (CsA+vit ADE) were higher than all other groups at 12 and 16 weeks.

9. Serum Creatinine Levels

Serum creatinine levels of Group A (CsA) at 4, 8, 12, and 16 weeks were higher than all other groups. In addition, the height at the 8th and 16th weeks is statistically significant. Group B (CsA+vit ADE) serum creatinine levels were significantly lower than group A (CsA) at 8 and 16 weeks. Serum creatinine levels of Group C (MMF) and Group D (MMF+vit ADE) remained at constant levels throughout the study.

10. Creatinine Clearance

Group A (CsA) 8th-week creatinine clearance was lower than group B (CsA+vit ADE), group C (MMF), and Group D, Group D (MMF+vit ADE) 12th-week creatinine clearance was significantly lower than group B (CsA+vit ADE) is significantly low. The creatinine clearance of group C (MMF) and group D (MMF+vit ADE) at weeks 8 and 16 were significantly higher than group A (CsA) and group B (CsA+vit ADE). In addition, the highest creatinine clearance measurements at week 16 were in Group C (MMF) and group D (MMF + vit ADE), the lowest measurements were in group A (CsA), group B (CsA + vit ADE), and group F (NAC + vit ADE).

11.a. TOS

The mean serum TOS values of the groups according to the weeks are shown in Table 1. Group F (NAC + vit ADE) mean serum TOS levels were higher than group A (CsA) and group D (MMF+vit ADE) at 4, 8, and 16 weeks, and group C (MMF) at 8 and 16 weeks. The mean TOS values of group E (vit ADE) at week 16 were significantly higher than group C (MMF) (Table 1).

11.b. TAS

The mean serum TOS values of the groups according to the weeks are given in Table 2. The mean serum TAS levels of group A (CsA), group C (MMF), group D (MMF+vit ADE), and group E (vit ADE) at week 16 were significantly higher compared to group F (NAC + vit ADE) (Table 2).

12. Histological Evaluation

In Table 3, the descriptive statistics of the groups are given as mean±SD. In addition, the p values obtained in the histopathological examinations of the groups are given in Table 4.

12.a. GSI

The lowest GSI value is in group E (vit ADE). GSI value of group E (vit ADE) was found to be significantly lower than group A (CsA), group D (MMF+vit ADE) and group F (NAC+vit ADE) (Tables 3 and 4).

12.b. Total damage score (TDS)

The lowest mean TDS was found in group E (vit ADE). TDS value of group E (vit ADE) was found to be significantly lower than group A (CsA), group D (MMF+vit ADE) and group F (NAC+vit ADE) (Tables 3 and 4).

12.c. IFS

The lowest mean IFS was detected in group D (MMF+vit ADE). Mean IFS of group A (CsA) versus group F (NAC+vit ADE), mean IFS of group B (CsA+vit ADE) and group D (MMF+vit ADE) versus group E (vit ADE) were found to be significantly low (Tables 3 and 4).

Table 1. Serum Total Oxidant Status (TOS) Levels (μmol/L). In comparisons between and within groups, only significant p values are shown.

WEEK	CsA (A)	CsA+Vit ADE (B)	MMF (C)	MMF+Vit ADE (D)	Vit ADE (E)	Vit ADE+NAC (F)	p (Intergroup)
0	19.01±9.33	33.56±26.01	22.63±5.89	24.56±8.24	21.63±2.68	19.11±8.00	p>0.05
4	16.66±3.97	20.06±4.25	20.67±9.88	16.99±2.95	15.53±2.67	29.86±16.16	A-F=0.04 B-E=0.02 D-F=0.02 E-F<0.01
8	21.87±8.70	21,48±6.80	22.28±7.80	16.40±3.71	23.06±7.58	32.54±15.21	A-F=0.04 C-F=0.03 D-F<0.01
12	37.57±12.36	38.40±15.31	33.25±14.2	24.00±8.88	34.81±21.4	36.43±27.19	p>0.05
16	17.25±5.28	29.66±18.55	17.01±3.68	21.28±6.00	25.83±11.27	40.79±21.25	A-F<0.01 C-E=0.03 C-F=0.02 D-F=0.01 E-F<0.01
p (in-group)	4-12=0.01 8-12 <0.01 12-16=0.01	p>0.05					

Table 2. Serum Total Oxidant Status (TAS) Levels (μmol/L). In comparisons between and within groups, only significant p values were shown

WEEK	CsA (A)	CsA+Vit ADE (B)	MMF (C)	MMF+Vit ADE (D)	Vit ADE (E)	Vit ADE+NAC (F)	p (intergroup)
0	3.03±0.03	2.79±0.46	3.00±0.13	2.70±0.35	2.94±0.24	2.93±0.29	p>0.05
4	2.96±0.28	2.86±0.28	3.03±0.18	2.75±0.48	2.79±0.43	2.30±0.97	p>0.05
8	2.33±0.91	2.80±0.17	2.47±0.76	2.82±0.32	2.53±0.76	2.14±0.88	p>0.05
12	2.13±0.77	2.54±0.33	2.65±0.44	2.92±0.30	2.31±0.62	2.29±1.08	p>0.05
16	3.08±0.13	2.94±0.52	2.98±0.31	2.98±0.21	2.92±0.33	2.22±0.82	A-F <0.01 C-F= 0.02 D-F= 0.01 E-F <0.01
p (in-group)	12-16=0.04	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05	

12.d. Degree of Staining with TGF-β

The lowest mean TGF-β score was found in group D (MMF+vit ADE). The mean of staining with TGF-β in group E (vit ADE) is significantly higher than group A (CsA), group C (MMF), group D (MMF+vit ADE) and group F (NAC+vit ADE) (Tables 3 and 4).

12.e. Degree of Staining with OPN

No significant difference detected between the groups in terms of the degree of staining with OPN.

Discussion

In previous studies using the adriamycin dosing regimen, renal function was well preserved up to week 16 then impaired, creatinine clearance decreased after 20 weeks.[3]

In our study, at the end of the 16th week, the lowest 24-hour urine protein was found in group B (CsA+vitamin ACE). The group with the highest serum creatinine level is group A (Csa), and the group with the lowest serum creatinine level is group D (MMF+vitamin ACE) after 20 weeks.

In a study performed in rats by generating passive Heyman nephritis, proteinuria and serum creatinine levels were compared by administering CsA and MMF. Although proteinuria decreased, serum creatinine levels were found to be higher in rats receiving CsA. In our study, serum creatinine was found to be significantly higher in the group receiving CsA compared to the other groups. However, both the decrease in GFR and decrease in serum albumin may have reduced proteinuria relatively. Again, consistent with this study, the lowest serum creatinine and creatinine clearance values were detected in group C (MMF).[10]

In an experimental study in which crescentic glomerulonephritis was generated in rats, a significant improvement

Table 3. Descriptive statistics of the histopathological examination data of the groups.

Histopathological examination	Group	Mean±SD
GSI	A (CsA)	0.3071±0.2317
	B (CsA+Vit ADE)	0.2125±0.1493
	C (MMF)	0.5750±0.7941
	D (MMF+Vit ADE)	0.3563±0.2382
	E (Vit ADE)	0.1313±0.0703
	F(Vit ADE+NAC)	0.7071±0.7155
TDS	A (CsA)	0.3071±0.23171
	B (CsA+Vit ADE)	0.2125±0.1493
	C (MMF)	0.5750±0.7941
	D (MMF+Vit ADE)	0.3563±0.2382
	E (Vit ADE)	0.1313±0.07039
	F(Vit ADE+NAC)	0.7071±0.7155
IFS	A (CsA)	0.4857±0.3387
	B (CsA+Vit ADE)	0.4250±0.2362
	C (MMF)	1.1375±1.5954
	D (MMF+Vit ADE)	0.6000±0.1690
	E (Vit ADE)	0.9000±0.3703
	F(Vit ADE+NAC)	2.0000±1.6901
TGF-β	A (CsA)	9.9714±2.3019
	B (CsA+Vit ADE)	11.7000±4.3504
	C (MMF)	11.5250±3.5370
	D (MMF+Vit ADE)	7.1625±5.7328
	E (Vit ADE)	16.1875±4.4144
	F(Vit ADE+NAC)	9.2571±3.2025
OPN	A (CsA)	19.7333±4.4410
	B (CsA+Vit ADE)	15.6000±7.8048
	C (MMF)	12.3000±7.0714
	D (MMF+Vit ADE)	16.1750±6.7588
	E (Vit ADE)	13.4625±4.8062
	F(Vit ADE+NAC)	16.0714±5.5616

was found in proteinuria with MMF administration. In our study, proteinuria was found to be higher at 4, 8 and 16 weeks and lower at 12 weeks in the group receiving MMF compared to the group receiving CsA.[18]

In our study, the lowest serum creatinine and optimal creatinine clearance levels were detected in group C (MMF). These data show that group C is the group in which renal damage has the least reflection on kidney functions. The lowest serum triglyceride levels were detected in group D (MMF+ADE) and the highest in groups A and B, which received CsA. This shows the increase in lipid peroxidation and ROS in rats receiving CsA and possible renal damage. The fact that serum cholesterol levels are at the lowest level suggests that group D (MMF+ADE) may be a good treatment option against the risk of steroid hypercholesterolemia.

In one study, rats were nephrectomized and kidney failure was created, and vitamins A, D and E were given to different groups. Although tissue damage decreased in all groups, the least tissue damage was observed in the group given vitamin E.[12]

Table 4. Results of analyzes of intergroup comparisons of histopathological data (only lines with significant difference between groups are given)

GROUPS	GSI	TDS	IFS	TGF-β	OPN
A - E	0.03	0.03	> 0.05	0.015	> 0.05
A - F	> 0.05	> 0.05	< 0.01	> 0.05	> 0.05
B - E	> 0.05	> 0.05	0.026	> 0.05	> 0.05
C - E	> 0.05	> 0.05	> 0.05	0.031	> 0.05
D - E	0.044	0.044	0.044	< 0.01	> 0.05
E - F	< 0.01	< 0.01	> 0.05	0,011	> 0.05

In our study, the renal damage score of group E (Vit ADE) was found to be significantly lower than groups A, D and F. The lowest serum creatinine level was detected in group D (MMF+ADE). In NS, kidney injury is associated with the severity of proteinuria. In this context, it is significant that the lowest 24-hour urine protein was detected in group B (CsA+vit ACE). Proteinuria was found to be higher in rats receiving MMF alone compared to those receiving MMF+vit ACE at 4, 8 and 16 weeks without significant difference. This finding shows that the positive contribution of adding vit ACE to the treatment can be questioned.

In the above-mentioned study, positive effects of vitamins (A, D, E) were observed by using them separately in nephrectomized rats. Again, although there are studies on the use of different antioxidants and vitamins separately together with CsA, no study was found in which all three vitamins were used together.[12]

In our study, no significant change was observed in TOS, which is an indicator of oxidative tissue damage, and TAS values, which give an idea about antioxidant levels, with the addition of vitamin ACE combination to CsA treatment. However, TAS values at the 16th week of the study showed a statistically significant increase in the group receiving only CsA.

NAC is the precursor of glutathione, one of the most important antioxidants. Glutathione is in high concentration in kidney tissue. NAC has entered into classical use in the treatment of radiocontrast nephropathy with its antioxidant and tissue damage reducing effects. In rats given adriamycin, its use with vitamin E significantly reduced nephrotoxicity. In another study, nephrotoxicity induced by administration of CsA to rats was significantly reduced by administration of NAC.[19-21]

In our study, in rats given NAC + vitamin ACE combination,

TOS was found to be significantly higher, TAS significantly lower, and creatinine clearance was lower. According to these data, it was evaluated that the combination of NAC + vitamin ACE did not contribute to the treatment in rats in which we created NS in the FSGS model with adriamycin.

In one of the similar studies, nephrotoxicity was created with gentamicin in rats. Rats were treated with a combination of NAC + vitamin ACE, and the values indicating kidney functions improved. In another study, rats with nephrotoxicity caused by cisplatin were treated with vitamin E, erythropoietin and NAC. According to the results of the study, only vitamin E among the agents reversed the increase in nitrite or nitrate levels from the findings of nephrotoxicity.[22,23]

It is possible that the difference in results between our study and the two studies is due to the difference in the designs of the studies. The results of the other study are compatible with our study. Although these studies are similar to ours, studies with NAC+vitamin ACE combination are very limited in the literature. This increases the original value of our work.

In one of two different studies, heyman's nephritis was created in rats, and in the other, NS in the FSGS model was created with adriamycin. In both studies, weight gain was detected in rats by week. However, the results of another study are in line with those obtained in our study. In the study, nephrotoxicity was induced by adriamycin in rats. According to the results obtained, the body weights of the rats increased with treatment with some agents, but decreased with others. Some of these changes are statistically significant.[24-26]

In our study, the 8th week weights of groups A, B and D were found to be significantly lower than their 4th week weights. In addition, when the body weights of group A and B rats receiving CsA were compared, it was evaluated that the combination of vitamin ACE added to CsA did not contribute positively to body weight.

A study was conducted to analyze the protective effect of the dipeptidyl peptidase-4 (DPP4) inhibitors sitagliptin and linagliptin from kidney toxicity. The rats that generated nephrotoxicity with doxorubicin were treated with these agents. In rats that developed nephrotoxicity, hypertension and proteinuria developed in a short time. Of the agents, only linagliptin reversed hypertension. In another study, rats induced nephrotoxicity with doxorubicin were treated with two different agents and their combinations. The results showed that high blood pressure resulting from nephrotoxicity did not decrease with combined therapy, and the use of an agent alone was more effective.[27,28]

In our study, 16th week systolic blood pressures of group E rats receiving vit ACE were found to be significantly higher. It has been observed that the use of vitamin ACE alone does not contribute to the treatment of NS. It was observed that systolic blood pressure values remained at normal levels, although there were differences between the groups.

The urine volumes of groups A and B receiving CsA at week 4 were found to be lower than those of group E (vit ADE) and group F (vit ADE+NAC). However, on the contrary, it was observed that the urine volumes of groups A and B increased at 12 weeks. In addition, while the urine volumes of group A receiving CsA at week 16 decreased compared to all other groups, no decrease was observed in group B receiving CsA + vit ACE. Addition of vitamin ACE to CsA made a positive contribution to urine volume. The lowest urine protein is also in group B. In the treatment of experimental NS, the addition of vitamin ACE to CsA resulted in both a decrease in proteinuria and an increase in urine output.

Proteinuria was also lowest in groups A and B who received CsA. Serum albumin levels were higher in groups D and E receiving MMF compared to groups receiving CsA. Although there were rat losses in the groups receiving CsA (2 from A, 3 from B), there was no loss of rats in the groups receiving MMF. In addition, TOS levels, which are indicators of oxidative tissue damage, were lower in groups receiving MMF than in other groups. As a result, mortality and morbidity rates of MMF treatment are lower than CsA in rats in which we created NS in the FSGS model with adriamycin.

TGF-β is a potent fibrogenic factor and is a determinant in the pathogenesis of glomerulosclerosis. TGF-β is a chemoattractant for fibroblasts and stimulates fibroblast proliferation and synthesis of extracellular matrix proteins in epithelial cells. TGF-β directly reduces the activity of metalloproteinases that break down extracellular matrix proteins. In addition, it inhibits the destruction of extracellular matrix proteins indirectly by increasing the effect of metalloproteinase tissue inhibitors.[29,30]

A study was conducted to examine the effect of gliotoxin on immunological and histological changes in the kidney using animal models. Accordingly, the increase in gliotoxin concentrations triggered TGF-β expression in the kidney. Immunohistochemical studies have revealed that increased TGF-β3 expression has a significant relationship between immunoreactive cell density and the number and size of lesions in the tissue. TGF-β expression was investigated in a study examining the role and underlying mechanism of lotensin in the late stages of chronic renal failure. It has been determined that lotensin protects against chronic kidney disease in rats through downregulation of TGF-β.[31,32]

In our study, although the lowest level of staining with TGF-β was not statistically significant, it was detected in the D group which received the combination of MMF and vitamin ACE.

In various studies, nephropathy was created in rats with different methods and the effects of vitamins A, D and E used in the treatment were analyzed. Accordingly, the GSI decreased significantly with the administration of vitamins A, D and E. Consistent with all these studies, in our study, the lowest GSI was found in group E rats receiving only vitamin ACE combination.[33,36]

The lowest IFS among all groups was found in group B who received a combination of vitamin ACE in addition to CsA. Renal histopathological scores (GSI, TDS, IFS) of group A and C rats receiving only CsA and MMF were significantly higher than the scores of groups B and D, in which vitamin ACE combination was added to CsA and MMF. According to these results, adding vitamin ACE combination to treatment reduces CsA nephrotoxicity and provides a renoprotective contribution to MMF treatment.

OPN is a 44 kilodalton phosphoprotein molecule normally secreted from the distal tubules. However, in renal pathologies with interstitial fibrosis (IgA nephropathy, lupus nephritis, membranous nephropathy, etc.), it is also secreted from the proximal tubules and is thought to be a chemotactic adhesive molecule for macrophages.[37]

It has been shown that kidney injury increases osteopontin expression in all kidney tubules and glomeruli. OPN is an early marker of tubular damage in experimental nephrotic syndrome. Puromycin aminonucleoside induces NS in rats. OPN mRNA was increased in the glomeruli of NS-induced rats with puromycin aminonucleoside. Consistent with these findings, a significant correlation was found between proteinuria and OPN expression in the kidney.[38-40]

In our study, the lowest staining levels with OPN in all groups were detected in group C rats receiving MMF, and the highest levels in group A rats receiving CsA, which is consistent with literature data. The OPN values of group A, which received only CsA, were higher than those of group B, which received a combination of vitamin ACE in addition to CsA. This shows the positive contribution of vitamins.

The use of vitamins A, D, and E in combination with CsA and MMF in experimental NS is not a common issue in the literature. In our study, vitamin ACE added to CsA treatment increased the antiproteinuric effect of CsA and reduced its nephrotoxicity. Vitamin ACE added to MMF contributed positively to biochemical and renal histopathological findings. New results can be obtained by conducting new studies on this subject.

Studies conducted in recent years have shown that the diagnosis of FSGS is made at an earlier age and steroid resistance is more common in cases. Despite efforts to treat it, the risk of ESRD is very high. CsA is the only agent with proven efficacy in the treatment of steroid-resistant NS, which is indispensable in organ transplant patients, and additional treatments are important to prevent possible side effects that limit its use. In our study, it is important to see that adding a combination of vitamin ACE to CsA resulted in a decrease in proteinuria and an increase in the amount of urine.[41]

CsA is nephrotoxic in the long term therefore, MMF is the most promising treatment alternative today. However, data on MMF are still insufficient and more studies are needed on its use in the treatment of FSGS. There are studies on the use of MMF in the treatment of FSGS in children, but these studies are limited and consist of data from selected patient groups. In our study, the lowest serum creatinine, triglyceride and cholesterol levels were detected in group D, which received a combination of MMF and vitamin ACE. Therefore, it can be said that adding a vitamin ACE combination to MMF treatment provides a positive contribution to kidney functions and lipid profile.[41-44]

Limitations

Due to the rules set by the ethics committee, a control group could not be formed. Urine and blood samples taken before the study were used as controls.

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Disclosures

Peer-review: Externally peer-reviewed.

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