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The effects of immunosuppressive agents on inflammatory response in septic rats

Research Article

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Abstract: Sepsis remains a serious clinical problems resulting in high morbidity and mortality. We aimed to investigate the inflammatory response in a septic rat model treated with immunomodulator agents. We used the cecal ligature and puncture model to establish septic peritonitis in rats. Male Wistar-Albino rats were randomized into groups of seven rats each and assigned different regimens: Tacrolimus 1 mg/kg/day, cyclosporin-A 5 mg/kg/day and methylprednisolone 15 mg/kg/day. These immunsuppressive agents were applied at the 6 and 48 hour intraperitoneally. The animals were euthanized after 6 and 48 hours and systemic parameters including, IL-2, IL-6, TNF-a, CRP, AST and creatinine were examined. Our study demonstrated that the experimental peritonitis model caused a meaningful rise in the values of systemic parameters. This was especially apparent for early-applied cyclosporin A; in addition, tacrolimus significantly decreased the levels both at the 6 and 48 hour. The excess immune response in complex sepsis treatment might be restrained using immunosuppressive agents administered early. Although additional supportive, comprehensive, experimental and clinical studies are still needed, this therapy model may prove to be an alternative for the future.

Keywords: *Inflammatory response • Immunosuppressive agents • Cyclosporine-A • Tacrolimus • Methylprednisolone*

1. Introduction

In the setting of documented infection, the systemic inflammatory response syndrome (SIRS) is called sepsis [1]. Sepsis is still a major cause of morbidity and mortality in intensive care units [2]: despite advanced pharmacological and surgical treatments, the procedures, mortality rate is as high as 40%–70% [3,4].

The SIRS results from a complex process that involves humoral and cellular responses and molecular episodes interacting in a complex cascade. The immunopathophysiology involves a variety of mediators, such as the tumor necrosis factor-alpha (TNF-α), interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), and interleukin-10 (IL-10) [5]. These primary inflammatory mediators induce the release of secondary inflammatory mediators, such as arachidonic acid metabolites, nitric oxide, and the platelet activating factor. The release of these inflammatory mediators results in destructive regional and systemic responses, and culminates in pathophysiological changes in various organs. This chain of events may terminate in multiple organ dysfunction syndrome (MODS). Recently, some studies conducted in patients with SIRS/Sepsis/MODS, the final immunologic response has proved to be either a pro-inflammatory response, an anti-inflammatory response, or both [6-9].

Cyclosporine-A (CsA) and tacrolimus decrease the production of various cytokines, such as IL-2 and interferon-γ, by acting specifically on lymphocytes. As they selectively suppress cellular immunity, they are called immunosuppressive agents. These agents are also used to prevent a rejection reaction in transplants, and also in the treatment of autoimmune diseases [10,11]. Glucocorticoids are also important immunosuppressive agents. However, when high-dose steroids are used for acute inflammation, especially for sepsis and septic

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shock treatment, secondary infections may develop. This is a clinical issue for which many different and contradicting opinions have been offered [12,13].

Since the uncontrolled pro-inflammatory mediators stimulate a SIRS / sepsis / MODS cascade at the onset of the inflammatory response, suppressing the release of these mediators may prevent the septic reaction. The aim of the study was to evaluate the effects of CsA, tacrolimus, and methylprednisolone on inflammatory mediators, such as IL-2, IL-6, and TNF-α, on the C-reactive protein (CRP) level, which is the non-specific indicator of inflammation, and on indirect organ insufficiency indicators such as serum aspartate aminotransferase (AST) and creatinine levels.

2. Material and Methods

2.1. Animals

We used 77 male Wistar-Albino conventional rats (6–8 weeks old, 230–300 g), purchased from the Experimental Animal Center of the Marmara University Medicine Faculty (Istanbul, Turkey). The rats were housed under specific pathogen-free conditions and acclimated to our facility for one week before the experiments. The experimental protocol was designed according to the Guiding Principles in the Care and Use of Animals endorsed by the Council of the American Physiological Society, and was approved by the local animal use ethics committee. Rats were kept in cages at 20 ± 2°C constant temperature, in 12-h light/dark cycles, and were fed with regular food and tap water from a drinking bottle.

2.2. Sepsis Model

Induction of peritonitis was performed in all rats using a cecal ligation and puncture (CLP). This method is based on a cost-effective, easy, and widely used model described previously [14,15]. Following overnight fasting, anesthesia for all animals was induced subcutaneously using 50 mg/kg ketamine HCl (Ketalar® 50 mg flk, Eczacıbaşı, Istanbul). This procedure was followed by a laparotomy with a 2-cm midline incision under aseptic conditions. The cecum was identified and ligated with 3-0 polypropylene (Prolene®, Ethicon, UK) from the antimesenteric side. The ligated cecum was punctured with a 25-gauge needle, squeezed to express a small amount of fecal material, and returned to and enclosed within the abdominal cavity in two layers using 4-0 silk sutures. Sham-operated rats underwent an identical laparotomy but did not undergo CLP. All rats were then resuscitated with subcutaneous 8 mL of normal saline solution. They received the same resuscitation load and no antibiotics or analgesics were used. No rats died during the study.

2.3. Experimental protocol

Rats were allocated into 11 groups of 7 rats each. Then 8 mL of intracardiac blood was drawn under anesthesia at preset times in these groups. At the end of the experimental protocol, all rats were euthanized with an overdose of sodium pentobarbital.

The drug doses administered in the present study were selected in accordance with previously conducted studies. Ellis RA et al have reported a statistically significant decrease in T-cell proliferation in 1 mg/kg/day tacrolimus-administered rats [11]. Fauvel H et al. [16] have administered 10 mg/kg CsA to septic rats to investigate its protective effect on myocardial dysfunction. The oral form of CsA is administered in two divided doses of 10–15 mg/kg/day to prevent the rejection reaction in organ transplants. The suggested concentrated intravenous CsA dose is about one-third the oral dose. In severe peritonitis and septic shock, short-duration corticosteroid therapy (1 to 2 days) and doses higher than 30 mg/kg are not recommended. In recent years, although long-duration low-dose continuous cortisone infusion therapy is suggested for peritonitis [17], we preferred high-dose cortisone for our study. Our aim was to repress the immunosupressive effect rather than the anti-inflammatory effect.

Control group (n=7): Intracardiac blood samples were taken and no further procedure was performed in this group.

Sham-operated group - 6th hour (n=7): Sampling at the 6th hour.

Sham-operated group - 48th hour (n=7): Sampling at the 48th hour.

Peritonitis group - 6th hour (n=7): Sampling at the 6th hour after CLP.

Peritonitis group - 48th hour (n=7): Sampling at the 48th hour.

Tacrolimus group - 6th hour (n=7): Soon after the CLP a 1-mg/kg single dose of tacrolimus (FK506, Prograf® injection 5 mg/mL, Fujisawa Healthcare, Deerfield, IL) was given intraperitoneally; a sampling was performed at the 6th hour.

Tacrolimus group - 48th hour (n=7): A 0.25-mg/kg dose of tacrolimus was given intraperitoneally 4 times at 12-h intervals for 36 hours (0–12th–24th–36th hours, total 1 mg/kg/day) and a sampling was performed at the 48th hour.

Cyclosporin A group - 6th hour (n=7): Soon after the CLP a 5-mg/kg single dose CsA (Sandimmun® injection 50 mg/mL, Novartis Pharma AG, Basel, Switzerland) was

given intraperitoneally and a sampling was performed at the 6th hour.

Cyclosporin A group - 48th hour (n=7): A 1.25 mg/kg CsA was given intraperitoneally 4 times at 12-h intervals for 36 hours (0–12th–24th–36th hours, total 5 mg/kg/day) and a sampling was performed at the 48th hour.

Methylprednisolone group - 6th hour (n=7): Soon after the CLP, a 15-mg/kg single dose of methylprednisolone (Prednol-L® injection 20 mg/mL, Mustafa Nevzat, Istanbul, Turkey) was given intraperitoneally; sampling was performed at the 6 hour.

Methylprednisolone group - 48th hour (n=7): A 3.75 mg/kg of methylprednisolone was given intraperitoneally 4 times at 12-h intervals for 36 hours (0–12th–24th–36th hours, total 15 mg/kg/day) and a sampling was performed at the 48 hour.

2.4. Evaluation

The blood samples extracted from the rats were centrifuged at 3000 revolutions/minute for 5 minutes. After separating the residual blood cells, the blood plasma was stored at -70 °C until analysis. The concentration of serum IL-2, IL-6, and TNF-α levels in the blood was determined by a standard sandwich enzyme-linked immunosorbent assay (ELISA) method with a rat TNF-α traditional ELİSA kit (Biosource Int, Ca, USA); with a rat IL-2 traditional ELİSA kit (Biosource Int, Ca, USA), and with rat IL-6 traditional kit (Biosource Int, Ca, USA) using the Medispec ESW 300 Elisa plate washer (Palmcity 72, USA), and with Humanreader, Human GmbH W 6204 (Taunustein, Germany) equipment. For the serum CRP measurement, the CRP traditional kit (CRPH, Beckman Coulter, Fullerton, California, USA) was used with the Beckman Array Protein System (Beckman Coulter, Fullerton, California, USA) equipment nefelometrically. The AST and creatinine serum levels were measured with an autoanalyser (Olympus AU 800, Tokyo, Japan).

2.5. Statistical Analysis

The data acquired from the study groups were evaluated via the Statistical Package for Social Sciences for Windows 11.0 (SPSS). The Mann Whitney U and Wilcoxon tests were used to evaluate the quantitative data. Data were expressed as mean ± S.E.M. The results were assessed in 95% confidence interval. All *p* values less than 0.05 were considered statistically significant.

3. Results

The evaluation of IL-2 levels showed that these levels were significantly increased in the sham group (p=0.02), but decreased in the tacrolimus group at the 6 and 48 hours (p=0.04) (Table 1). When the correlation between the groups was analyzed, it was determined that the IL-2 levels had increased significantly in the peritonitis group compared with the control at the 6th and 48th hours (p=0.0001) (Table 2). In all drug groups, these levels were lower than the peritonitis group at the 6th hour (p=0.0001), but they remained lower only in the tacrolimus and CsA groups at the 48th hour (p=0.0001) (Table 2). The IL-2 values were significantly lower in the CsA and tacrolimus group than those in the methylprednisolone group at the 6th and 48th hour (p=0.01, p=0.0001, respectively, for both).

IL-6 levels were significantly lower at the 48th hour in all groups except the control (p<0.05) (Table 1). However, the comparison between peritonitis and control groups showed that these levels were significantly higher in the peritonitis group (p=00001) (Table 2). When we compared all treatment groups with each other, we found that all drugs decreased the IL-6 levels at the 6th and 48th hour in the presence of peritonitis (p=0.0001 for both). Tacrolimus decreased the IL-6 values more significantly than methylprednisolone at the 6th hour; a similar result was found for the CsA at the 6th and 48th hours (p=0.0001 for both) (Table 2).

We determined a decrease of TNF-α levels in the sham and peritonitis groups at the 48th hour (p=0.02 for both) (Table 1). The relationship among the groups showed us that the TNF-α levels had increased significantly in the peritonitis compared with the control at either time point (p=0.0001) (Table 2). All drugs decreased the TNF-α levels significantly compared with the peritonitis group at the 6th and 48th hours (p=0.0001 for both). There were no statistically significant differences between the drugs in terms of alteration of TNF-α levels (p >0.05).

CRP values were significantly higher in the peritonitis group at the 48th hour (p=0.02) (Table 1). When the mice were evaluated within a given group, the CRP was significantly higher in the peritonitis group than those in the control group at the 6th and 48th hours (p=0.0001). Tacrolimus and CsA reduced the CRP levels in the peritonitis group at the 6th hour (p=0.0001), while these levels were also reduced significantly at the 48th hour using all three drugs (p=0.0001 for all three) (Table 2).

The induction of peritonitis resulted in a significant increase in serum AST levels by the time of progress (p=0.02), but other groups did not show a similar result (p>0.05) (Table 1). Serum AST levels were significantly

Table 1. Values of the parameters of groups (mean ± SEM).

Groups	IL-2			IL-6			TNF-α			CRP			AST			CREATININ		
	6h	48h	p[£]	6h	48h	p[£]	6h	48h	p[£]	6h	48h	p[£]	6h	48h	p[£]	6h	48 h	p[£]
Control	13.03± 1.65			1.8 ± 0.1			5.0 ± 2.0			2.9 ± 0.5			127.7 ± 29.4			0.6 ± 0.1		
Sham	10.94±0.74	13.51±2.03	0.02*	2.5±0.2	1.7±0.3	0.02*	23.0±3.9	4.8±2	0.02*	5.8±0.5	2.8±0.5	0.02*	219.9±40.5	237.7±36.4	0.40	0.7±0.04	0.7±0.1	0.40
Peritonitis	25.6±2.17	23.13±1.7	0.06	290±94.7	64.1±17.8	0.02*	465.6±371.1	72.7±31.7	0.02*	8.1±0.9	15.8±1.4	0.02*	261.1±107.1	896.8±213.9	0.02*	0.7±0.1	0.6±0.1	0.09
Tacrolimus	3.81±2.25	1.9±0.71	0.04*	117.8±14.5	1.9±0.18	0.02*	4.5±2.39	4.2±4.4	0.74	1.9±1.1	2.9±1.6	0.13	181.7±51.2	191±20.2	0.35	0.6±0.1	0.7±0.2	0.40
Cyclosporin-A	5.59±5.36	3.21±2.52	0.31	50.1±35.1	1±0	0.04*	9.1±15.9	9.6±11.3	0.87	2.6±1.8	3.02±1.7	0.87	190.7±31.8	210.1±52.5	0.24	0.6±0.2	0.7±0.1	0.61
Methylprednisolone	17.11±6.08	19.14±6.07	0.31	54.2±26.9	10.5±12.4	0.02*	13.0±13.5	2.4±0.7	0.08	5.8± .9	3±1	0.18	216.7±43.3	211.3±28.2	0.73	0.7±0.1	0.7±0.1	0.74

AST=Aspartat amino transpherase, CRP= C-reactive protein, IL-2=interleukin-2, IL-6=interleukin-6, TNF-α=Tumor necrosis factor-alpha, SEM= Standard error of mean, h.= hour, p[£] = p values between 6th and 48th hours.

higher in all groups than those of the control group at the 6th hour ($p<0.05$), and higher levels were more significant in the sham and methylprednisolone groups ($p=0.0001$). We found that none of the drugs had decreased the AST levels at the 6th hour ($p>0.05$). However, serum AST levels were significantly higher in all groups than those in the control group at the 48th hour ($p=0.0001$); the drugs lowered these levels significantly compared to the peritonitis group at a given time ($p=0.0001$) (Table 2).

There was no significant alteration in creatinin levels at the 6th and 48th hours in any group, and in addtion, these levels were not different between the peritonitis and control groups ($p>0.05$) (Table 1). Tacrolimus and CsA decreased creatinine only at the 6th hour compared with the peritonitis ($p=0.02$, $p=0.03$, respectively) (Table 2).

4. Discussion

In our study, we determined that the experimental peritonitis model led to significantly higher levels of serum IL-2, IL-6, TNF-α, CRP, AST and creatinine. In addition, when immunosuppressive agents such as CsA, tacrolimus and methylprednisolone were administered in the early periods, CsA and tacrolimus most particularly led to significant decreases in the relevant parameters, both at the 6th and 48th hours.

After the invasion of pathogenic microorganisms, a complex cascade of events is initiated in which primarily polymorphonuclear leukocytes, which play a crucial role in host defence, migrate to the infectious site and kill microorganisms by releasing bactericidal agents such as cytokines. Cytokines are signalling glycoproteins that regulate numerous cellular functions: they are important factors in cell differentiation, proliferation, and activation, and also modulate proinflammatory and anti-inflammatory responses to allow the host to react appropriately to pathogens. Cytokines play a critical role in host defence, wound healing, and other host functions; there is a considerable level of regulation between them that is required for an appropriate immune response to sepsis. If either proinflammatory or anti-inflammatory cytokines are allowed to predominate without appropriate regulation, there is a high potential for tissue and organ damage [18-20]. Numerous trials have documented increases in the plasma concentrations of cytokines in patients with severe sepsis, including TNF-α, IL-2 and IL-6 [21].

Cyclosporine A and tacrolimus are immunosuppressive macrolides as well as powerful and efficient lymphocyte inhibitors. They also inhibit IL-2 release from T lymphocytes. Calcineurine is a

Table 2. The statistical significance of parameters (p values) between the groups.

		IL- 2		IL-6		TNF-α		CRP		AST		CREATININ	
		6th h	48th h	6th h	48th h	6th h	48th h	6th h	48th h	6th h	48th h	6th h	48th h
Control	Sham	0.01	0.61	0.0001	0.22	0.0001	0.85	0.0001	0.48	0.0001	0.0001	0.52	0.20
	Peritonitis	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.03	0.0001	0.08	0.95
	Tacrolimus	0.0001	0.0001	0.0001	0.16	0.14	0.14	0.14	0.85	0.03	0.0001	0.75	0.48
	Cyclosporin - A	0.02	0.0001	0.01	0.0001	0.04	0.65	0.85	0.85	0.01	0.0001	0.37	0.37
	Methylprednisolone	0.18	0.04	0.0001	0.05	0.99	0.0001	0.18	0.14	0.0001	0.0001	0.41	0.22
Peritonitis	Tacrolimus	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.22	0.0001	0.02	0.40
	Cyclosporin – A	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.16	0.0001	0.03	0.36
	Methylprednisolone	0.0001	0.12	0.0001	0.0001	0.0001	0.0001	0.05	0.0001	0.41	0.0001	0.37	0.12
Tacrolimus	Cyclosporin - A	0.70	0.75	0.0001	0.0001	0.44	0.12	0.57	0.75	0.28	0.34	0.52	0.95
	Methylprednisolone	0.0001	0.0001	0.0001	0.37	0.65	0.70	0.06	0.99	0.02	0.37	0.37	0.56
Cyclosporin - A	Methylprednisolone	0.01	0.0001	0.95	0.0001	0.65	0.90	0.14	0.95	0.22	0.99	0.16	0.56

AST = Aspartat amino transpherase, CRP = C-reactive protein, IL-2 = interleukin-2, IL-6 = interleukin-6, TNF-α = Tumor necrosis factor-alpha

phosphatase that is the target of CsA and tacrolimus; it plays a critical role in T cell activation and cytokine gene expression. Cyclosporine A and tacrolimus inhibit the catalytic activity of calcineurine, forming a complex with cyclophilin and FKBP12 (protein binding tacrolimus), respectively. T cell and cytokine activation occur by activating the T cell nuclear factor (NFAT), which belongs to the transcriptional regulator family. Following de-phosphorylation of NFAT, it is translocated into the nucleus, at which time various cytokines, such as IL-2, IL-4, TNF-α, are released [22]. Our strategy was to use the suppressive effect of CsA and tacrolimus on the immune system; the results were successful. In CsA and tacrolimus-administered rats, we detected that immune cytokines such as TNF-α, IL-2 and IL-6 were suppressed in a statistically significant way.

Spijkstra et al. [23] have reported that, in patients with septic shock, cytokines bind to cortisol-binding receptors. This increases the glucocorticoid resistance and decreases the arterial blood pressure, resulting in a relative insufficiency. Total glucocorticoid resistance causes in vitro IL-2 increase in the monocytes of patients with peritonitis. In patients with septic shock, the indirect indicator of the developing glucocorticoid resistance is an increased plasma cortisone level as a result of prolonged hydrocortisone usage. Bone et al. [17] have found that hydrocortisone infusion (10 μg/hour) provided clinical and hemodynamic stability in patients with septic shock; these authors also reported that high physiological doses of cortisol could regulate the immune response. Meduri et al. [24] determined that, while methylprednisolone decreases the serum level of pro-inflammatory cytokines such as IL-6 and IL-8 in patients with acute respiratory distress, hydrocortisone decreases their serum level in septic shock.

One of the important mediators appearing in sepsis is TNF-α. Cannon et al. [25] have reported that TNF-α levels increase in animals and humans with sepsis. Viallon et al. [26] have concluded that the TNF-α level in serum and in the peritoneal liquid of patients with spontaneous bacterial peritonitis was higher than in patients with non-bacterial peritonitis (patients with sterile acid liquid) in a statistically significant manner. Saito et al. [15] found that the TNF-α level was higher than the sham group at the 6th hour, but it decreased to the sham group values at the 24th hour. Garcia-Criado et al. [27] have reported that tacrolimus inhibited IL-1 and TNF-α production in the rat liver ischemia/reperfusion injury model. Fauvel et al. [16] have determined that calcineurine inhibitors regulated cell death stimulated by TNF-α and nitric oxide. They emphasized that CsA and tacrolimus are powerful inhibitors of rat liver cell cytotoxity induced by TNF-α. We discovered that TNF-α levels were at their highest at the 6th hour, and although these levels decreased at the 48th hour, they remained very high when compared with the control and drug-administered groups. Tacrolimus maintained TNF-α values at the control group value both at the 6th and 48th hours. Although the values of the other drug-administered groups were meaningfully and dramatically much lower than the peritonitis group at the 6th and 48th hours, there were not different from those of the tacrolimus group. This situation has led us to conclude that both tacrolimus and CsA, in particular, could inhibit the pro-inflammatory response by preventing TNF-α levels from increasing.

Koshika et al. [28] have reported that an increase in serum TNF-α level accompanied with high serum IL-2 is an indicator of high mortality rates in septic shock patients. Furthermore, tacrolimus has the potential to inhibit not only IL-2, but also IL-1 and TNF-α production, and could suppress septic shock and MODS progression by

inhibiting the organization of monocytes, macrophages, and lymphocytes. In our study, we found that IL-2 levels had dramatically increased in the peritonitis group when compared with the control both at the 6th and 48th hours, but there were no statistically significant differences between the 6th and 48th hour values. In the drug-administered groups, although methylprednisolone administration had significantly decreased IL-2 levels at the 6th hour, this effect did not continue into the 48th hour. Tacrolimus and CsA had decreased IL-2 levels dramatically when compared with the control group. This situation probably resulted because tacrolimus and CsA had suppressed T-helper cells from releasing IL-2. These values were compatible with concurrent studies where IL-2 decreased with TNF-α [27,28].

IL-6 is one of the pro-inflammatory cytokines that is produced by various cells such as monocyte, macrophage, lymphocyte, fibroblast and endothelial cells. Giannoudis et al. [29] have demonstrated that serum IL-6 concentration has an important role in tissue injury and in SIRS, and is significantly increased in patients with severe trauma. In one report, it has been found that in patients with a high Apache II and organ insufficiency score, IL-6 levels were significantly increased. Concurrently, these IL-6 levels showed a correlation to hypothermia and mortality [30]. In the same study, while IL-6 serum levels were higher in the peritonitis than the sham group at the 6th hour, these levels were dramatically higher at the 24th hour compared with the sham group. In the present study, IL-6 levels were significantly increased in the peritonitis group when compared with the control, especially at the 6th hour; and although there was a temporary regression at the 48th hour, this increase was still meaningful. Though three drug administrations produced severe regression in IL-6 levels at the 6th hour, this effect was more evident in the CsA and methylprednisolone groups. But at the 48th hour, especially with the tacrolimus and CsA, values decreased to the control group levels. The IL-6 level, which is accepted as a good indicator of mortality and prognosis, could be reduced with tacrolimus, CsA, and methylprednisolone administration. Furthermore, this effect could be more pronounced at the 48th hour, especially with CsA and tacrolimus.

C-reactive protein is an acute phase plasma protein in which the plasma level rises quickly and dramatically in acute injuries such as infection and trauma. In some trials, it has been demonstrated that CRP levels rise in a statistically significant manner and are an indicator of inflammation [30,31]. Kuster et al. [32] have reported that, while the diagnostic sensitivity of CRP before the clinical appearance of peritonitis is 18%, its sensitivity is 93% at the time of the clinical diagnosis. Since the serum CRP level was high in the peritonitis group in our study, this indicated that we had constructed an efficient sepsis model. Although it is a nonspecific indicator, we concluded that CRP is a sensitive systemic marker of inflammation and tissue damage. In the drug-administered groups, especially in the tacrolimus and CsA groups, serum CRP levels were maintained close to a normal value, and the levels were very low when compared with the peritonitis group. This led us to believe that these immunosuppressive agents were quite successful in depressing not only the immune system but also the inflammatory response of the body.

Some studies indicate that cytokines, such as TNF-α, induced rat liver cell cytotoxicity, and the AST level increased at the 12th hour and reaching its maximum level at the 36th hour [31,33]. In our study, AST levels were used as the indicator of liver toxicity and organ failure. The AST levels in the peritonitis group had increased in a statistically significant way at the 48th hour. The levels in the other groups were also higher than the those of the control, but they were not elevated compated with the values of the sham group in any of the drug-administered groups. This situation led us to suggest that liver injury, which is the result of sepsis, could be prevented or decreased by tacrolimus, CsA, and methylprednisolone.

Another organ injury parameter is creatinine. Edremitlioglu et al. [34] have found that the plasma creatinine level was higher in the sepsis. Creatinine levels maintained normal values both in the peritonitis and the drug-administered groups in our study. This led us to believe that sepsis needs a long time to disrupt the renal function. We also assumed that tacrolimus and CsA, which are considered to be nephrotoxic drugs, could not disrupt renal function with applied doses at the early stage.

We conclude that the early application of immunosuppressive therapy decreased the pro-inflammatory response and restrained organ failure in our experimental peritonitis model. Further investigation with more comprehensive experimental and clinical studies may be necessary to identify the exact doses, timing, and duration of treatment to prevent the devastating effect of sepsis.

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