

Is tri-iodothyronine a better choice than activated protein C in sepsis treatment?

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ABSTRACT

BACKGROUND: Sepsis can be defined as a life-threatening organ dysfunction due to a dysregulated host response to infection. In sepsis, the coagulation cascade is activated and the balance shifts to the procoagulant side. Recently, the use of protein C is proposed for the treatment of sepsis. Another therapeutic agent that has been intensively studied is tri-iodothyronine.

METHODS: This study aimed to compare the effects of activated protein C and tri-iodothyronine, which are administered at a single dose to sepsis-induced rats at the late phase. Leukocyte, platelet, hemoglobin and antithrombin-III concentrations and histopathological changes in the small intestine, liver and lung were evaluated at 24 hours.

RESULTS: Single-dose intraperitoneal recombinant human APC (activated protein C) has a partial curative effect on hematological parameters in the late phase, while it is possible to state that it has significant therapeutic effects on hepatic and intestinal tissues, but more remarkably on the lung tissue. Tri-iodothyronine is also considered to be used for the treatment and has a strong potential to be a therapeutic agent.

CONCLUSION: We observed that the T3 hormone has significantly limited and reduced the sepsis-related damage to hepatic and intestinal tissues, but especially the lung tissue. Tri-iodothyronine can be a good alternative to APC, which is partially allowed due to high cost and complication of bleeding in the treatment of sepsis.

Keywords: Activated protein C; sepsis; tri-iodothyronine.

INTRODUCTION

Sepsis was first defined in 1991 as the inflammatory response of the host and it has been addressed within this context until very recently. After pathophysiology of sepsis is better understood, the definition is revised as “an infection-related response of the host that is not only limited to pro- and anti-inflammatory response but also contains modifications in non-immunological pathways, such as cardiovascular, autonomic, neurological, hormonal, metabolic and coagulation systems”.^[1]

Sepsis was defined in the Sepsis III Conference held in 2016: A life-threatening organ dysfunction due to a dysregulated host response to infection. On the other hand, septic shock is defined as circulatory and cellular/metabolic abnormalities

that cause a severe increase in the mortality in the presence of sepsis.^[1]

Septic shock is accompanied by severe hypovolemia, vasodilatation and cardiac dysfunction. Septic shock is a severe hypoperfusion of tissue characterized by cytopathic hypoxia and microcirculation that are induced by systemic inflammatory response secondary to infectious etiologies.^[2]

The primary goal of treatment is to eliminate the infectious agent that leads to sepsis. On the other hand, the recent novel treatment options aim to support the first-line treatment of sepsis by inhibiting or eliminating the immune modulation and their adverse effects over the inflammatory cells, cytokines, antibodies, complements and coagulation cascades that play a role in the inflammation. However, no agent could

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be invented to date, which would pose the expected effect on these pathophysiological changes, is low cost, has favorable side effect profile and can be clinically used in a larger patient population.

In sepsis, the coagulation cascade is activated, while native anticoagulant mechanisms are suppressed and the balance shifts to the procoagulant side. Protein C, Protein S and Antithrombin III are natural anticoagulants and their plasma concentrations decrease in sepsis. Recently, the use of Protein C, one of the major natural anticoagulants, is proposed for the treatment of sepsis. Protein C plays a role in the regulation of the fibrinolytic activity by converting into the activated Protein C in the presence of thrombomodulin – thrombin complex that is found in the endothelium. However, Protein C is insufficiently converted into the active form in sepsis and plasma concentrations decreased below 40% of the physiological limits. Therefore, it was considered that APC replacement in sepsis would help the fibrinolytic effects of APC and also its inhibitory effects on the production of proinflammatory cytokines from monocytes, and it had been subject to numerous clinical and experimental studies.^[3]

Another therapeutic agent that was intensively studied is tri-iodothyronine that acts in the regulation of genes, which play a role in the systemic cellular inflammatory response. Low thyroid hormone concentration is called low tri-iodothyronine (T3) syndrome. Low T2 syndrome is characterized by a reduction in concentrations of Total T3, Free T3 and Total T4. Free T4 or TSH concentrations can be low or within physiological ranges. This condition is called non-thyroidal disease syndrome that is characterized by changes in serum thyroid hormone concentrations without accompanying thyroid disease. In sepsis, low T3 concentrations are usually secondary to severe acute inflammation. The primary factor is the dysfunction of the hypothalamo-pituitary axis.^[4] The changes in thyroid function tests of patients with septic shock had led to many investigators to the view whether thyroid hormone replacement would be beneficial in the treatment of sepsis.

Our study used the polymicrobial sepsis model that has signs and symptoms proven similar to intra-abdominal sepsis and is induced by caecal ligation and puncture (CLP) procedure.^[5] According to this model, the early period implies two hours to ten hours after the CLP (hypodynamic phase), while the late phase is regarded as 16 to 20 hours after the procedure (hyperdynamic phase). This study aimed to compare the effects of activated protein C (Xigris) and tri-iodothyronine (Thyrotardin Inject), which are administered at a single dose to sepsis-induced rats at the late phase (24 hours), in the treatment of sepsis. Complete blood count and plasma AT-III concentrations are analysed, and the histopathological changes are reviewed in the lungs, liver and small intestine.

MATERIALS AND METHODS

Study Groups and Study Design

Our study is approved by the Ethical Committee of Laboratory Animals at Hacettepe University and it is conducted in Laboratory Animals and Surgical Research Unit of School of Medicine at Surgical Research Unit of School of Medicine at Hacettepe University. Each study group included 30 female Sprague Dawley rats that weighed 250 to 300 gr. All rats were fed standard rat diet (chow) and tap water until the study day. They were not allowed to eat or drink at the night of the study.

Surgical Procedure

Rats were anesthetized by administering Xylazin Hydrochloride (Rompun®) at dose of 5 mg/kg and Ketamine Hydrochloride (Ketalar®) at a dose of 50 mg/kg through the intraperitoneal route at the day of this study. The anterior abdominal wall was shaved and scrubbed with betadine solution. Median incision was carried out at sterile conditions and the abdominal cavity was exposed. Rats were divided into five main sub-groups. The distribution of the groups is shown below.

I. Control Group (n=6): In this group of rats, a median incision (approx. 1.5 to 2 cm) was carried out to expose the abdominal cavity. Caecum was identified and it was normal in the exploration; it was reduced to the abdominal cavity. The abdominal wall was closed with 3/0 atraumatic silk suture with non-continuous technique.

II. CLP Group (n=6): This group of rats is denominated the group of septic rats and a median incision (approx. 1.5 to 2 cm.) was made. Caecum was removed and it was tied with 4/0 free silk using the Wichterman method, such that intestinal continuity was not hindered.

Caecum was punctured at two points with 18 Gauge syringe. Caecum was mildly milked and a certain amount of feces was discharged. Caecum was reduced into the abdominal cavity thereafter and the abdominal wall was closed with 3/0 atraumatic silk suture with non-continuous technique.

III. APC (Activated Protein C Replacement) Group (n=6): For this group of rats, caecal ligation and puncture were performed, as is the case with the CLP Group. These rats were administered Drotrecogin Alfa (Xigris®, Lilly) at a dose of 100 µg/kg through intraperitoneal route 1 hour after the procedure.

IV. Tri-iodothyronine (T3 replacement) Group (n=6): This group of rats was administered Tri-iodothyronine (Thyrotardin® Inject N Henning, Berlin GMBH, Germany) at a dose of 0.4 µg/100g through intraperitoneal route one hour after the CLP procedure.

V. Combined Treatment (APC+T3 Replacement)

Group (n=6): This group of rats was administered Drotrecogin Alfa (Xigris®, Lilly) at dose of 100 µg/kg and Tri-iodothyronine (Thyrotardin® Inject N Henning, Berlin GMBH, Germany) at dose of 0.4 µg/100g, both through intraperitoneal group, 1 hour after CLP.

After surgical procedures were performed and replacement therapies were over, rats of all groups were administered 4 ml of heated isotonic sodium chloride through the subcutaneous route. Wound sites were closed with Tetracycline ointment (Terramycin®, Pfizer). They were placed in the cages, where they were allowed to eat and drink sufficiently until the sampling procedure was performed.

Analysis of Hemoglobin, Leukocyte and Platelet Counts (24 hours)

Rats of all groups were sacrificed by intra-cardiac blood drawing with re-laparotomy for samplings following the anesthesia with XylazineHidroklorid (Rompun®) at the dose of 5 mg/kg and Ketamine Hydrochloride at the dose of 50 mg/kg at 24 hours. Blood samples were added to complete blood count tubes that contained 180 µl of 0.4% EDTA in 2 cc and sent to the Hematology Laboratory of Ankara Numune Teaching and Research Laboratory.

Antithrombin-III Sampling (24 Hours)

Four millimetres of the intracardiac blood sampled from rats of all groups were added to the tubes that contained citrate and centrifuged (3000 rpm, 30 minutes). Blood samples were centrifuged and the plasma was taken with automated pipettes; plasma samples were added to Eppendorf tubes and sent to the Hematology Laboratory for Antithrombin III assay.

Tissue Specimens of Liver, Lung and Intestines (24 hours)

After blood samples were drawn, the liver, lungs and small intestines were biopsied in all rats. For this end, the left lobe of the liver, left lobe of the lung and approximately 1.5-cm piece of the terminal ileum were biopsied with standard procedure for each rat and tissue pieces were placed in bottles that contained formol. They were sent to Pathology Laboratory Ankara Numune Teaching and Research Hospital. Specimens were stained with Haematoxylin-Eosin and examined by a pathologist who was blinded to the study groups under a light microscope.

Statistical Analysis

The data were analyzed using non-parametric Kruskal Wallis and Mann-Whitney U tests using SPSS 13.0 Windows Chicago Illinois software; a p-value <0.05 was regarded significant at a 95% confidence interval.

RESULTS

Leukocyte Concentrations (24 Hours)

Leukocyte concentration 24 hours after CLP was 4566/µl in the control group, 3133/µl in the septic rat group, 3266/µl in the Activated Protein C replacement group, 6083/µl in the tri-iodothyronine replacement group and 5050/µl in the combined (Activated Protein C + Tri-iodothyronine) replacement group (Fig. 1). When leukocyte concentrations of all groups were compared, the leukocyte concentrations of the septic group and the APC group were significantly lower than the leukocyte concentrations of the control group (p=0.006 and p=0.013, respectively). Leukocyte concentrations of the T3 replacement group were significantly higher than the leukocyte concentrations of the control group (p=0.003), while no significant difference was observed between the leukocyte concentrations of the combined treatment group and the control group. When replacement groups were compared with the septic group concerning leukocyte concentrations, no significant difference was observed between the APC group and the septic group, while leukocyte concentrations were significantly higher in rats of the T3 group and the combined treatment group (APC/T3) than the rats of the CLP Group (p<0.001 and p=0.001, respectively).

Platelet Concentrations (24 Hours)

Platelet concentrations at 24 hours were 870.10³/µl, 496.10³/µl, 357.10³/µl, 291.10³/µl and 328.10³/µl in control group, CLP group, APC replacement group, T3 replacement group and combined treatment group, respectively (Fig. 2). Platelet concentrations of the control group were higher than platelet concentrations of all other groups (p<0.001). Platelet concentrations of the septic group (CLP) measured significantly higher than platelet concentrations of the replacement groups (APC, T3, APC/T3) (p=0.037, p=0.005 and p=0.007, respectively). No significant difference was observed in intergroup comparisons of the replacement groups.

Hemoglobin Concentrations (24 Hours)

Hemoglobin concentrations at 24 hours measured 12.72 g/dl in the control group, 14.03 g/dl in the septic rat group, 14.68 g/dl in the activated Protein C replacement group, 14.43 g/dl in the tri-iodothyronine replacement group and 12.02 g/dl in the combined replacement group (Fig. 3). Considering intergroup comparisons of hemoglobin concentrations, no significant difference was observed between hemoglobin concentrations of the control group and hemoglobin concentrations of all other treatment groups. Only hemoglobin concentrations of the activated Protein C replacement group were significantly higher than hemoglobin concentrations of the combined treatment group (p=0.03).

Antithrombin-III Concentrations (24 Hours)

Anti-thrombin concentrations at 24 hours measured 147.08 in the control group, 111.98 in the CLP rat group, 108.92 in

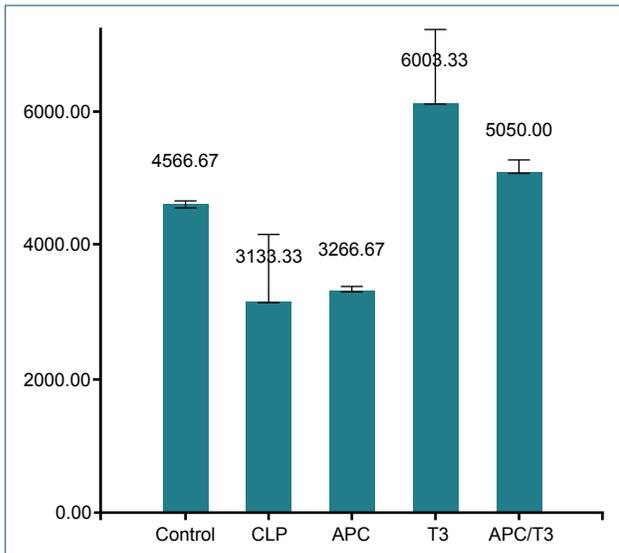


Figure 1. Columns show the late phase (24 h) leukocyte concentrations for each group.

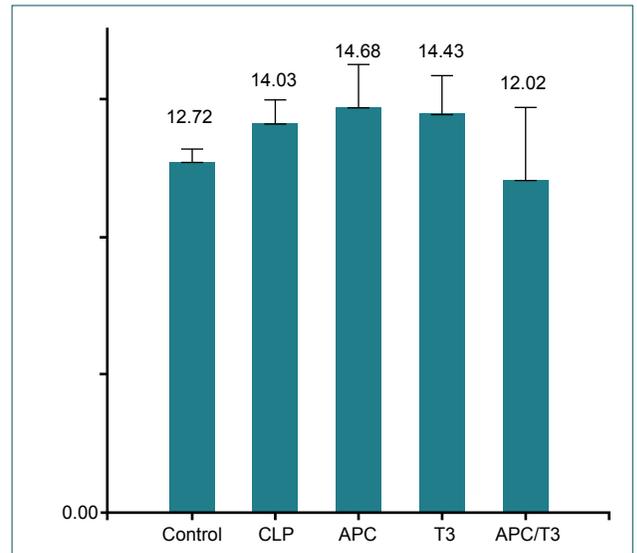


Figure 3. Columns show the late phase (24 h) hemoglobin concentrations for each group.

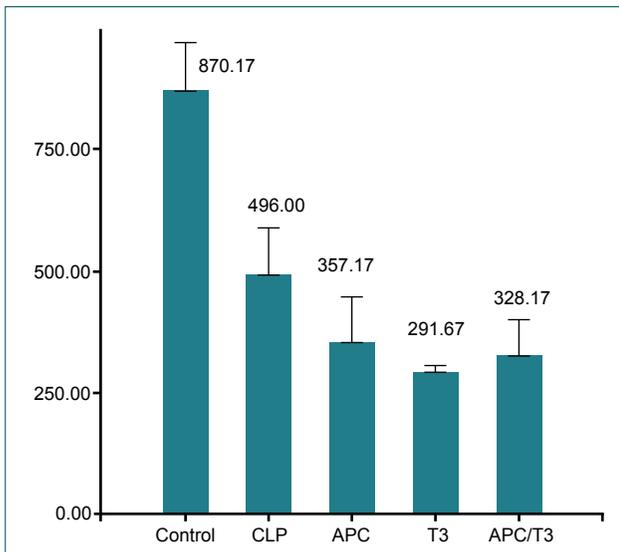


Figure 2. Columns show the late phase (24 h) platelet concentrations for each group.

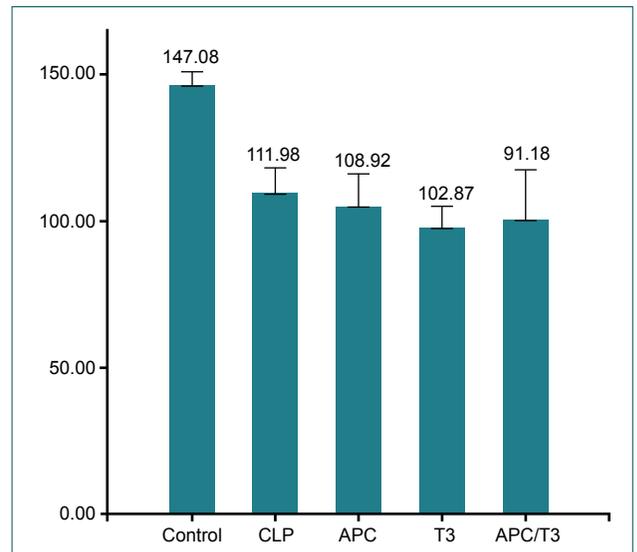


Figure 4. Columns show the late phase (24 h) antithrombin-III concentrations for each group.

the activated Protein C replacement group, 102.87 in the tri-iodothyronine replacement group and 91.18 in the combined replacement group (Fig. 4). Antithrombin concentrations of the control group were significantly lower than that of other groups ($p < 0.001$). There was no significant difference between rats of activated Protein C and CLP groups concerning Antithrombin III concentrations, while antithrombin III concentrations of the CLP Group were significantly higher than antithrombin III concentrations of the tri-iodothyronine group ($p = 0.002$) and the combined replacement group ($p = 0.001$).

Histopathological Changes in the Small Intestines (24 hours)
 In the histopathological examination of the intestinal tissue, pieces biopsied 24 hours after CLP, focal necrosis was noted in the intestinal tissue pieces of three rats in the CLP group

(50%), while it was accompanied by microvascular thrombosis in one of these rats (17%). Diffuse congestion was identified in intestinal tissue specimens of the other three rats (50%). No significant change was noted in intestinal tissue specimens of the activated Protein C group, while ischemic changes were observed only in one rat in the Tri-iodothyronine group (17%). Considering rats of the combined treatment group, no significant histopathological change was observed, as is the case with rats of the control group. Figure 5 shows the histopathological changes in the small intestine.

Histopathological Changes in Lung Tissue (24 Hours)

When the histopathological changes were reviewed in the

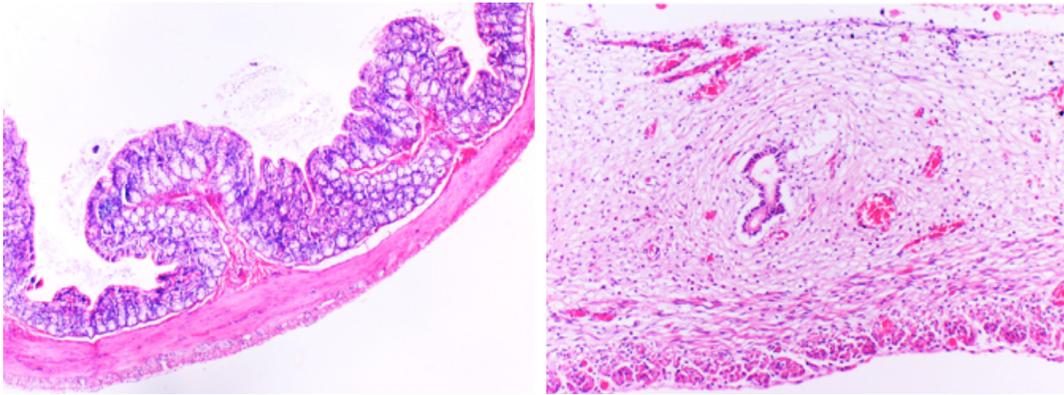


Figure 5. First image shows normal intestine tissue and the second image shows congested intestine tissue, 20X, Hematoxylin and Eosin (H&E).

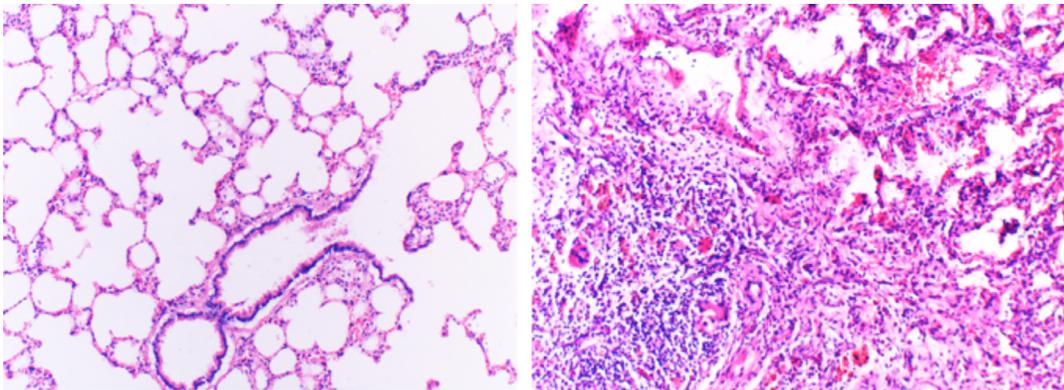


Figure 6. First image shows normal lung tissue and the second image shows infiltrated lung tissue by PMN, 20X, Hematoxylin and Eosin (H&E).

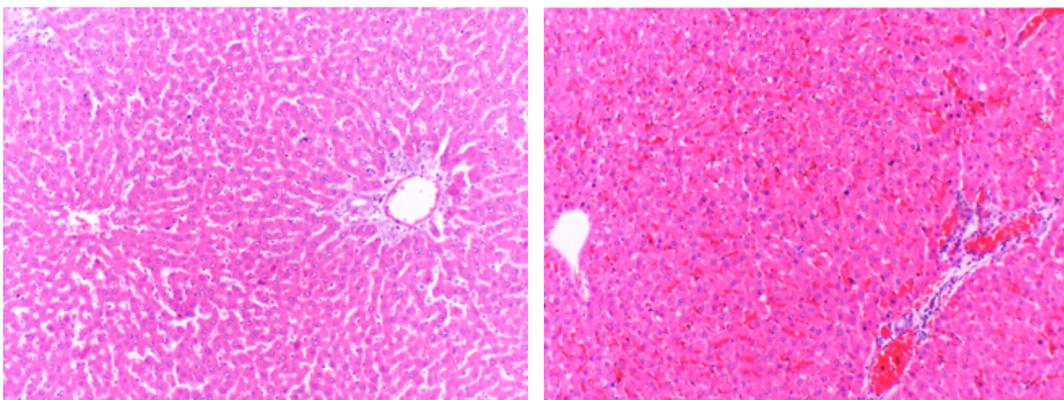


Figure 7. First image shows normal liver tissue and the second one shows congested liver tissue, 20X, Hematoxylin and Eosin (H&E).

lung tissue that was biopsied 24 hours after CLP, significant congestion developed in all rats of the CLP group (100%) in comparison with rats of the control group that were all normal, while the congestion was associated with polymorphonuclear leukocyte infiltration in the lung tissue of two rats (34%). Mild congestion was noted in the lung tissue of one rat in the activated Protein C group (17%), two rats in the Tri-iodothyronine replacement group (34%) and two rats in the combined treatment group (34%). Polymorphonuclear leukocyte infiltration was seen in none of the rats in replacement

groups (0%). Figure 6 shows the histopathological changes in lung tissue.

Histopathological Changes in Liver Tissue (24 Hours)

When liver tissue specimens were examined, significant congestion was noted in five rats of the CLP group (85%), while congestion was associated with necrotic foci in three rats (50%). Congestion was observed only in two rats of the ac-

tivated Protein C group (34%), while mild congestion was identified in three rats of the tri-iodothyronine replacement group (50%) and two rats of the combined replacement group (34%). Figure 7 shows the histopathological difference between normal and congested liver tissue.

DISCUSSION

Sepsis is defined as a life-threatening organ dysfunction secondary to the dysregulated host response due to the infection.^[1] Although numerous measures were taken and introduced into the practice in the recent past to improve the management of sepsis and treatment outcomes, mortality is still high in patients with severe sepsis and septic shock. A study reported that the incidence of severe sepsis is 148/100,000 and the mortality is 26 percent.^[6] It was also reported that severe sepsis progressed to septic shock approximately by 25 percent and as a result, the mortality rate increased to 37.2 percent.^[7]

Activation of the coagulation system is the major factor that is broadly responsible for such adverse outcomes. DIC is a clinical picture that is characterized by systemic, intravascular activation of the coagulation pathways. It is reported that DIC may develop in 35% of patients with septic shock. Activation of the Protein C system poses anticoagulant, anti-inflammatory, anti-apoptotic and endothelial barrier stabilizing effects under physiological conditions.^[8] After protein C, the main component, binds to endothelial protein C receptor (EPCR), it is activated by the thrombomodulin-thrombin complex.^[9] Dysregulation of the Protein C system is the most important sign of the coagulopathy that is related to septic shock. Reduced protein C concentrations are linked to high morbidity and mortality.^[10] Although the significant role of APC in sepsis is known, measurement of APC in patients with sepsis has not yet been specified in routine clinical practices.

Complex etiopathogenesis of sepsis leads to important problems in clinical trials and treatment-oriented investigations. Since the physiological response of humans differs from animals, it is not easy to use agents with efficiency proven in investigational studies in the clinical practice due to comorbid pathologies and differences in duration of treatment and dosage.^[11]

Numerous agents with efficiency proven in investigational trials were used in the clinical practice, particularly including Antilipid, Interleukin-1 antagonists, platelet-activating factor inhibitors, immunoglobulins, ibuprofen, high-dose corticosteroids and antithrombin III, but the expected success could not be gained. Recombinant human activated Protein C was the first and the only agent with proven success that was approved by the FDA (Food and Drug Administration) for the treatment of sepsis in early 2001 and it was introduced into the clinical practice. Subsequently, early goal-directed therapy and low-dose corticosteroid replacement were started to be used in the treatment of sepsis.^[12]

Our study used an experimental polymicrobial sepsis model that is induced by CLP and strikingly mimicking the intra-abdominal sepsis secondary to intestinal perforation, a condition commonly diagnosed in surgery clinics. In the experimental sepsis model that is induced with caecal ligation and puncture, early (hyperdynamic) phase initiates 2 to 10 hours after CLP, while late (hypodynamic phase) emerges 16 to 20 hours after CLP.^[13,14] Our study is conducted in the hypodynamic phase of the sepsis. However, two reasons are considered for low leukocyte counts in rats that were administered activated protein C. First, the suppressive effect of the activated protein C on the relation between leukocytes and endothelium in the early phase of the sepsis disappears in the late phase, or the other estimation is the insufficient effect of the single-dose activated Protein C replacement to maintain leukocyte concentrations.^[15]

Numerous studies have demonstrated that leukocyte and platelet concentrations of the host are indicators commonly used to reveal out the inflammation and coagulation abnormalities in sepsis.^[8] When leukocyte concentrations were measured 24 hours after CLP, they were significantly lower in rats of CLP and APC (activated protein C) groups in comparison with the control group, while leukocyte concentrations were significantly higher in rats of tri-iodothyronine and combined replacement groups than rats of the CLP group. When all replacement groups were taken into consideration, leukocyte concentrations were significantly higher only in rats of the tri-iodothyronine group than the rats of the control group. Given that leucopenia is among the laboratory diagnosis criteria for sepsis, low leukocyte counts in rats of the CLP group is regarded as an evidence that sepsis could be induced in this model; endothelial adhesion of leukocytes, increased endothelial permeability and migration of leukocytes into the tissue are the potential mechanisms behind the low leukocyte concentrations.

It is demonstrated that serum T3 concentrations are related to the mortality in the septic shock. Yildizdaş et al.^[16] have shown a significant reduction in plasma tT3, tT4, fT3 and fT4 concentrations in pediatric patients with septic shock and patients managed in the intensive care unit relative to adult and pediatric patients without septic shock. Similar results were revealed out in a series of 20 adult cases.^[17] Higher Apache 2 scores and poorer prognosis are reported for patients with low serum fT3 and fT4 concentrations.^[18] Moreover, exogenous T3 replacement may reduce the mortality in surgery patients with sepsis.^[19]

Thyroid hormone and immune functions are closely interlinked. Thyroid hormone plays an important role in myeloperoxidase antimicrobial system by supplying iodine. Fernandez and Videla demonstrated high NADPH and myeloperoxidase activities in rats that were fed T3.^[20] On the other hand, Inan et al.^[5] showed that thyroid hormone replacement increased the phagocytic activity of neutrophils in septic animal mod-

els. Thyroid hormone replacement influences the activation of other immune cells in addition to neutrophils. Alamino reported that T3 replacement increased activation and expansion in cytotoxic T lymphocytes.^[21] Our study also determined that leukocyte concentrations could be significantly maintained in rats of tri-iodothyronine replacement – a finding that matches the literature. We consider that the boosting effect of T3 hormone on cellular and humoral immunity is responsible for this result.^[22]

Reduced platelet concentration is regarded as an important parameter to prove the presence of sepsis. Thrombocytopenia that is observed in bacterial sepsis can be related with Disseminated Intravascular Coagulation, but lack of any relationship is also likely. There are two causes for thrombocytopenia that is not associated with DIC. First, bacteria or endotoxins may inhibit production of platelets; this possibility may be proven by a study conducted by Ayala et al.^[23] who demonstrated the reduction of viable cell counts in the bone marrow in sepsis that is induced by CLP.

The other cause is the immunological platelet damage. Increased plasma concentration of platelet-associated immunoglobulin G in patients with thrombocytopenic sepsis is the evidence for the immune damage.^[24] In our study, a significant reduction was observed in platelet concentrations of all groups, excluding the control group. It is observed that the protective effect of the activated protein C and tri-iodothyronine replacement on platelet concentrations in the early phase could not be maintained in the late phase. Moreover, platelet concentrations of the replacement groups were even significantly lower than platelet concentrations of the CLP group.

We consider the potent coagulative effects of more than one factor, especially including decreased peripheral blood supply along with endothelial damage and dysfunction, for the inability to maintain platelet concentrations in the late phase of sepsis by single-dose replacements of both activated protein C and tri-iodothyronine. This fact clarifies that replacement therapies should be maintained also in the late phase.

Hemoglobin concentration is the other hematological parameter that is analyzed in this study. No significant reduction is observed in hemoglobin concentrations when septic rat and replacement groups are compared with the control group. We can specify, in the light of these findings, that lack of reduction in hemoglobin concentrations in the control group at 24 hours is a factor, which prevents the onset of severe tissue hypoxia in the non-lethal CLP model.

AT-III concentrations were significantly low in our study groups in comparison with the control group. When compared with the replacement groups, AT-III concentrations were significantly high in the CLP group. Contrary to other results, Chapital et al.^[25] conducted a study, which evidenced the therapeutic effect of tri-iodothyronine on AT-III concen-

trations in sepsis; authors have reported that tri-iodothyronine was intravenously infused at a dose of 3 ng per hour for postoperative 24 hours and AT-III concentrations were measured at 24 hours. In our study, tri-iodothyronine was administered at a single dose of 0.4 µg/100 g/day, as already reported by other studies.⁵ We consider the differences in doses and measurement intervals for the deviation from the literature concerning therapeutic effects of activated protein C and tri-iodothyronine, alone or in combination, on AT-III concentrations in the late phase of the polymicrobial sepsis induced by CLP.

A clinical study conducted by Eisele et al.^[26] verifies the conclusion of our investigation study that although T3 replacement has no therapeutic effect on plasma AT-III concentrations, it has significantly limited and reduced the tissue damage caused by the sepsis over anti-inflammatory effects of APC and T3 rather than over the coagulation cascade.

Oxygen consumption decreased in the intestinal and hepatic tissues at the late phase of the sepsis and therefore, both tissues become vulnerable to hypoxia. Moreover, polymicrobial sepsis impairs the intestinal barrier; reduces the functional capillary density and leads to apoptosis of the lymphoid tissue. Bacterial and endotoxin translocation are a result of damage to the intestinal barrier and it also triggers a humoral and cellular immune response in the host. The intestinal barrier accounts for 80% of the humoral immunity and 50% of the cellular immunity. Polymicrobial sepsis activates the release of cytokines, such as TNF-α and IL-6, from the intestinal tissue. Role of the intestinal tissue in sepsis is not confined to being an organ that can synthesize cytokines. The relation between the intestinal tissue and Kupffer cells of the liver is far more important in the systemic response to the sepsis. Kupfer cells produce 90% of macrophages in the reticuloendothelial system and it is the major source of the proinflammatory cytokines.^[27]

When histopathological changes are examined in the intestinal tissue specimens of rats in the CLP Group, diffuse congestion is noted in all specimens, while microvascular thrombosis or necrotic foci are identified in a significant part of them. In both activated protein C and tri-iodothyronine replacement groups, histopathological changes were significantly different from the changes identified in septic rats. Necrotic foci or microvascular thrombosis was observed in none of the intestinal tissue specimens in rats of the replacement groups.

Healing effects of the activated protein C on the intestinal tissue may be explained with anti-apoptotic, anti-inflammatory and anti-coagulant effects in lymphoid and endothelial tissues. A study conducted by Yang et al.^[28] reported that in an experimental polymicrobial sepsis model, tri-iodothyronine replacement reduced the permeability of the intestinal tissue significantly and prevented potential structural and functional damages to the intestinal tissue secondary to the sepsis,

but further studies are warranted to understand this protective mechanism better. Tri-iodothyronine probably acts by increasing the synthesis of preventive substances in the intestinal epithelial cells and boosting the endurance of the intestinal epithelium to the oxidative stress. This view is also supported by studies that demonstrated atrophy in intestinal epithelial cells and a reduction in mucosal DNA and protein in hypothyroidism.

When hepatic tissue specimens of our study groups were examined, diffuse congestion was observed in liver tissues of all rats in the CLP group, while necrotic foci were noted in a part of them; congestion was milder and there was no focal necrosis in tissue specimens of the rats in APC, T3 and APC/T3 groups. The changes in liver tissues of septic rats were matching to the findings reported in the literature.^[29] Increased permeability secondary to endothelial function was considered responsible for the diffuse congestion in rats of the CLP Group, while preventive effects of the activated protein C may cover suppression of proinflammatory cytokines and limitation of microvascular coagulation.^[30] As already expressed here, the preventive effects of tri-iodothyronine on the liver tissue may include the regulatory effects on cellular and humoral mechanisms.^[31]

An experimental study conducted by Lotková et al.^[32] demonstrated that tri-iodothyronine replacement activates the mitochondrial glycerophosphate cytochrome-c reductase, which is responsible for regeneration in the liver of rats, and it increased the hepatic regeneration in rats that were undergone partial liver resection. It is possible to think that this effect may also develop in rats with sepsis and the regulatory effect of tri-iodothyronine on cellular metabolism and proliferation may play a role in the regeneration.

The most significant sepsis-related organ failure is probably ARDS that develops in the lung tissue. ARDS develops approximately in 20–40% of patients with sepsis.^[33] The sepsis-related changes in the lung tissue include activation of leukocytes and monocytes along with alveolar macrophages, pulmonary edema secondary to increased permeability following the interaction of leukocytes and endothelium and the onset of microvascular thrombosis.^[34] It is observed that the activity of nuclear factor kappa B (NFκB), which has a proven trigger role in the inflammatory response to the infection, increased in alveolar macrophages after experimental polymicrobial sepsis. The induction of this increase by TNF-α indicates that TNF-α may also be held responsible for the development of ARDS.^[35] When the literature is reviewed, it is revealed out that these cytokines impair the synthesis of surfactant on the alveolar epithelium and decrease pulmonary compliance.

The diffuse congestion and leukocytic infiltration in rats of the septic group match the findings of the literature.^[36] It is

observed that the histopathological changes in the lung tissue secondary to the polymicrobial sepsis are significantly limited in rats of the replacement group. We believe that the preventive effects of the activated protein C in the lung include anti-inflammatory effects and suppression of the leukocytic damage to the endothelium. On the other hand, increased pulmonary compliance, improved synthesis of surfactant and regulation of the systemic cellular response are the preventive effects of tri-iodothyronine.^[31]

Single-dose intraperitoneal recombinant human APC, which has a proven effect in the treatment of sepsis, has a partial curative effect on hematological parameters in the late phase, while it is possible to mention that it has significant therapeutic effects on hepatic and intestinal tissues, but more remarkably the lung tissue.^[29,37]

Tri-iodothyronine is also considered to be used for the treatment and has a strong potential to be a therapeutic agent, as it plays a role in the expression of genes that regulate the cellular immunity and metabolism; in our study, we observed that T3 hormone has significantly limited and reduced the sepsis-related damage to hepatic and intestinal tissues, but especially the lung tissue.

Conclusion

Considering the ease of administration and low treatment cost, we believe that tri-iodothyronine will be a good alternative to APC, which is partially allowed due to high cost and complication of bleeding in treatment of sepsis. However, further studies with larger populations and different doses are warranted to have the final word, as already mentioned by all studies on sepsis.

Thanks

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Conflict of interest: None declared.

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DENEYSEL ÇALIŞMA - ÖZET

Sepsis tedavisinde triiyodotironin aktive protein C'ye göre daha iyi bir seçenek midir?

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AMAÇ: Sepsis, enfeksiyona karşı konağın göstermiş olduğu disregüle yanıtı bağlı gelişen hayatı tehdit edici bir organ disfonksiyonu olarak tanımlanabilir. Sepsiste, koagülasyon kaskadı aktive olur ve denge prokoagülan tarafa doğru kayar. Protein C'nin sepsis tedavisinde kullanımını araştıran yakın tarihli çalışmalar mevcuttur. Ayrıca diğer bir terapötik ajan olan triiyodotironin hakkında da çalışmalar yapılmıştır.

GEREÇ VE YÖNTEM: Çalışmamızda sepsis oluşturulmuş sıçanlarda geç dönemde tek doz aktive protein C ve triiyodotironin uygulamasının etkileri karşılaştırıldı. Uygulama sonrası 24. saatte lökosit, platelet, hemoglobin ve antitrombin-III konsantrasyonları ile ince bağırsak, karaciğer ve akciğerdeki histopatolojik değişiklikler değerlendirildi.

BULGULAR: Intraperitoneal tek doz rekombinant insan APC (aktive protein C) uygulamasının geç fazda hematolojik parametrelerde küratif etkileri olduğu ve başta akciğer olmak üzere hepatik ve ince barsak dokusunda da anlamlı terapötik etkilerinin olabileceği görülmüştür. Triiyodotironin ise sepsis tedavisinde kullanılabilecek önemli bir terapötik ajan olarak değerlendirildi.

TARTIŞMA: Çalışmamızda T3 hormonunun özellikle akciğer dokusu olmak üzere karaciğer ve ince bağırsak dokusu üzerinde sepsis bağlı hasarı sınırladığı veya azalttığı gözlenmiştir. Triiyodotironin daha az maliyet ve kanama riskiyle sepsis tedavisinde APC'ye iyi bir alternatif olabileceği kanaatindeyiz.

Anahtar sözcükler: Aktive protein C; sepsis; triiyodotironin.

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