Protein C, Protein S and Antithrombin III Levels in A Rabbit Sepsis Model

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Abstract: The consumption of coagulation inhibitors will augment coagulation triggered excessively in sepsis. The determination of levels of these inhibitors may open new substitution treatment schedules to overcome the plateau of the achieved improvement ratio with antimicrobial treatment. Staphylococcus aureus ATCC 25923, 1x10^9 per kg day for two days was administered intravenously to 30 albino rabbits to induce sepsis. Clinical and hematological signs were determined and sepsis was determined with blood culture. The complete blood count, protein C, protein S and antithrombin III levels were examined at the baseline and after sepsis development. The mean proportions of baseline to sepsis levels of protein C, protein S and antithrombin III were 69.3%, 56.5% and 117% respectively. The differences between the levels before and after sepsis were significant for protein C (p<0.001) and protein S (p<0.05). Although there was a slight increase in antithrombin III levels, the difference was not significant. Our study suggests that replacement therapy in sepsis was rational for consumption of protein C and protein S, but not for antithrombin III.

Key Words: protein C, protein S, antithrombin III, sepsis.

Introduction

Although antimicrobial treatments were developed in the last decade, sepsis still has a high mortality, especially when it has been complicated by shock and multiple organ system failure (1, 2). Because the improvement of prognosis with antibacterial treatment has reached a plateau, attention is focused on controlling the exclusively triggered defense mechanisms, and thus inhibitor systems have gained importance. It has been shown that treatment modalities targeting these systems may decrease mortality (1).

Sepsis causes the consumption of coagulation factors, platelets and fibrinolytics and inhibitor factors. Then both bleeding and thrombus tendencies occur. It is thought that coagulation plays an important role in organ failure in sepsis (2, 3). If this assumption is true, the correction of coagulation abnormality may improve the prognosis of sepsis. Protein C, protein S and antithrombin III are important in the control of coagulation and now, the purification methods from plasma are sufficient for commercial marketing. For this reason, the determinations of these inhibitors will help us to understand their role in sepsis and plan inhibitor therapy at optimal priority, sequence and time.

Materials and methods

Thirty albino rabbits, provided by the Medical Research Center of Ondokuz Mayas University, of average weight 0.5 kg were used. Staphylococcus Aureus ATCC 25923, 1x10^9 per kg day for two days was administered intravenously to the rabbits to induce sepsis. Clinical septic findings, fever, toxic granulation in blood smears and an increase in band/total neutrophil ratio were detected. Sepsis was determined by blood cultures. The complete blood count, protein C (Staclot protein C, clotting assay), protein S (Staclot protein S, clotting assay) and antithrombin III (Turbox, AT III) levels were determined at the baseline and after sepsis development.

Results

The mean proportions of baseline to sepsis levels of protein C, protein S and antithrombin III were 69.3%,...
56.5% and 117% respectively. The differences between the baseline and sepsis levels of protein C (p<0.001) and protein S (p<0.05) were significant. Although there was a slight increase in antithrombin III levels, the difference was not significant (Table 1). For the statistical analysis, the paired student t–test was used.

Table 1. Protein C, protein S and antithrombin III values in rabbits at baseline and after sepsis development (Mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Baseline (%)</th>
<th>After Sepsis (%)</th>
<th>After Sepsis / Baseline</th>
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<tbody>
<tr>
<td>Protein C</td>
<td>98.3 ± 9.8</td>
<td>68.1 ± 7.8</td>
<td>69.3</td>
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<tr>
<td>Protein S</td>
<td>101.0 ± 10.2</td>
<td>57.1 ± 8.3</td>
<td>56.5</td>
</tr>
<tr>
<td>Antithrombin III</td>
<td>94.2 ± 16.2</td>
<td>110.2 ± 19.4</td>
<td>117.0</td>
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Discussion

Many relationships have been established between sepsis and coagulation. Recent studies indicate that the activation of coagulation is mediated mainly, if not exclusively, through the expression of tissue factor on mononuclear cells in blood and endothelial cells (1). In sepsis, bacterial products and endotoxin and cytokines stimulate these cells. The administration of cytokines to animals or humans induces activation of complements and coagulation (4, 5). The activation of coagulation leads to consumption of both coagulation factors and inhibitors. The inhibitors are affected by different mechanisms: in vitro it has been shown that cytokines such as tumor necrosis factor, interleukin-1 and endotoxin reduce thrombomodulin expression (6, 7). Increased levels of C4bBP due to acute phase response will displace the equilibrium between protein S and C4bBP to the complex and inactive form of protein S. However, interleukin-6 also upregulates protein S expression (8). The liberated neutrophil elastase by activated leukocytes also appears to inactivate protein S (9).

There are limited numbers of animal studies about the relationships between sepsis and consumption of coagulation inhibitors. Taylor et al., have shown that activated protein C infusion administered at lethal doses of escherichia coli was able to prevent the shock response with generalized intravascular coagulation and fatal organ damage that follows such endotoxin doses in baboons (10). Other experiments on baboons performed by these authors also indicate that inhibition of either protein C or protein S activity converts the acute phase response to DIC and organ damage (11). Although these experiments are still preliminary, they strongly suggest an important protective role of protein C pathway in limiting damage due to inflammation. Our study confirmed protein C and S deficiency in sepsis but antithrombin III levels slightly increased. We found that antithrombin III replacement therapy in sepsis is controversial and its indications must be reevaluated (12).

In human studies, depressed protein C levels have been associated with sepsis (2), sepsis induced acute lung injury (13) and meningococcal septicemia (14). Decreased antithrombin III levels have been found in patients with sepsis (2), septic shock (15, 16), sepsis induced ARDS (17) and meningococcal septicemia (13, 18). Antithrombin III levels were found to be correlated with the sepsis or DIC score (19). Involvement of coagulation cascade in the pathogenesis of sepsis offers several possibilities for intervention that are complementary to the therapeutic maneuvers targeted at neutralizing the activity of endotoxin or cytokines. The coagulation system is regulated in vivo by inhibitors, notably protein C, protein S and antithrombin III. Meningococcemia was at the extreme point of the relationship between sepsis and coagulation abnormality because of the presence of infectious purpura fulminans. In a pediatric study, protein C administration reduced mortality (20). With other agents, useful effects on sepsis may be expected. The combined use of inhibitors may also be more effective.

In conclusion, our animal study confirmed that sepsis causes the consumption of coagulation inhibitors. The decreases in protein C levels were also confirmed in human studies. Protein C replacement therapy may be required to prevent thrombotic events and infectious purpura fulminans. Human studies should be performed concerning the decreases in protein C levels. The substitution therapy for antithrombin III may be rational only when low antithrombin III levels have been determined in a patient.

References


