Soluble Cell Adhesion Molecules and Parameters of Lipoprotein Metabolism in Patients with Severe Burns
F Rassoul¹, V Richter¹, C Kistner², D Wisser², B Reichert²

ABSTRACT

Background: The role of leukocyte adhesion molecules in patients with burns and their relationship to other parameters of inflammation and lipid metabolism is only recently beginning to be explored. Therefore, we investigated the temporal changes in the levels of soluble cell adhesion molecules and other parameters of inflammation and lipoprotein metabolism in patients with thermal injury. Materials and Methods: The serum levels of soluble adhesion molecules, intercellular cell adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), and sE-selectin, C-reactive protein (CRP) and fibrinogen in seven patients with severe burns over a 30-day period were measured to determine the involvement of these factors in the pathophysiology of severe burns. Serum levels of sICAM-1, sVCAM-1 and sE-selectin were determined by ELISA. Furthermore, total cholesterol, high-density lipoprotein cholesterol (HDL chol), low-density lipoprotein cholesterol (LDL chol) and triglycerides (TG) were measured.

Results: Blood levels of sICAM-1, sVCAM-1, CRP and fibrinogen increased with maximum values six days after thermal injury. In contrast, serum levels of sE-selectin were elevated two days after thermal injury. The sICAM-1, sVCAM-1 and sE-selectin levels correlated significantly with both the CRP and the fibrinogen levels. Plasma total cholesterol, HDL cholesterol and LDL cholesterol decreased with minimum values four days after thermal injury. Furthermore, an increase of triglyceride levels was observed.

Conclusion: The observed inflammatory response of soluble cell adhesion molecules could be useful in monitoring endothelial activation immediately following thermal injury. Further studies involving a larger number of patients with burns should help to clarify the extent to which measured parameters, especially the temporal changes of sCAMs, could be relevant in assessing the morbidity of patients with thermal injury.

Moléculas de Adhesión Celular Soluble y Parámetros del Metabolismo de la Lipoproteína en Pacientes con Quemaduras Severas
F Rassoul¹, V Richter¹, C Kistner², D Wisser², B Reichert²

RESUMEN

Antecedentes: El papel de las moléculas de adhesión leucocitaria en pacientes con quemaduras y su relación con otros parámetros de inflamación y metabolismo de lípidos ha comenzado a ser explorados sólo recientemente. Por lo tanto, investigamos los cambios temporales en los niveles de las moléculas de adhesión celular solubles y otros parámetros de inflamación y metabolismo de las lipoproteínas en pacientes con daños térmicos.

Materiales y métodos: Los niveles de suero de las moléculas de adhesión solubles, las moléculas 1 de adhesión intracelular (sICAM-1), las moléculas 1 de adhesión celular vascular (sVCAM-1) y sE-selectina, la proteína reactiva C (CRP), y el fibrinógeno en siete pacientes con quemaduras severas en un periodo de 30 días, fueron medidas a fin de determinar la participación de estos factores en la
INTRODUCTION

Activation of endothelial cells with increased expression and release of leukocyte adhesion molecules and changes of lipid-protein metabolism are involved in the pathology of burns. However, knowledge of the time sequence of changes of both parameters of inflammation and lipid metabolism in patients with severe burns is incomplete. In the present study, the temporal changes in the levels of soluble cell adhesion molecules, C-reactive protein, fibrinogen and parameters of lipid metabolism in patients with thermal injury were investigated. The mechanisms of leukocyte migration and accumulation in inflammatory joint tissues initially require adhesion to and migration through the vascular endothelium and then through synovial connective tissue. The binding of circulating leukocytes to vascular endothelial cells and their subsequent transendothelial migration into the subendothelial space are mediated by inducible cell adhesion molecules (CAMs) expressed on the surface of endothelial cell space (1–3). Upregulation of CAMs is accompanied by the release of soluble forms of adhesion molecules into the bloodstream. Therefore, increased plasma levels of soluble CAMs (sCAMs) have been suggested as markers of elevated CAM expression (1, 4, 5). Soluble CAM levels are elevated in subjects with cardiovascular and inflammatory diseases (6–8).

The role of levels of sCAMs as predictors of mortality in burn and critically ill patients is only recently beginning to be explored. Furthermore, their relationship to other parameters of inflammation and of lipid metabolism is not known. However, several results suggest that inflammatory cytokines and CAMs are strongly involved in the pathology of burns (9, 10).

In the current study, we aimed to further characterize the time sequence of changes of sCAMs in patients with burns. We measured levels of the soluble cell adhesion molecules, intercellular cell adhesion molecule-1 (sICAM-1, CD54), vascular cell adhesion molecule-1 (sVCAM-1, CD106) and sE-selectin (CD62E). Furthermore, we determined the concentrations of CRP, fibrinogen, total serum cholesterol, triglycerides, HDL cholesterol, nonHDL cholesterol and LDL cholesterol in dependence on time after thermal injury.

SUBJECTS AND METHODS

Patients

This work was performed as a pilot study on patients with burns admitted to the Burn Unit at Nuremberg Clinics (Nuremberg, Germany). The characteristics of the seven patients included in the study are shown in Table 1. The total burn surface area (TBSA) was between 15 and 45%. Venous blood samples in the frame of diagnostics were taken at 0, 2, 4, 6, 8, 18 and 30 days after thermal injury.

Measurements

Serum levels of soluble ICAM-1, soluble VCAM-1 and soluble E-selectin were determined by the use of monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA; R & D Systems, Europ Ltd., United Kingdom). All samples were tested in duplicate. Both interassay and intra-assay coefficients of variation were less than 5%. C-reactive protein levels in serum samples were determined quantitatively by an immunoturbidimetric assay (Tina-quant®CRP, Roche Diagnostic, Mannheim, Germany). Fibrinogen was measured in citrate plasma according to a modification of the Clauss method. The investigations were carried out as a routine diagnostic procedure. Serum concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL cholesterol) and triglycerides (TG) were measured by enzymatic reactions according to established procedures using commercially available test kits (Roche Dagnostic Systems – Reftotron 74 F autoanalyzer, Mannheim, Germany). The inter- and intra-assay coefficients of the methods are less than 5%. The quality control was performed with the control material Precinorm U (Roche Diagnostics, Mannheim, Germany). LDL cholesterol was calculated according to the Friedewald formula (11).
Statistical analysis
Data were analysed with SPSS (Statistical package for the Social Science Inc. Chicago Illinois) for Windows. Data are expressed as means ± SD to test changes in dependence on time after thermal injury; comparisons were performed using the Mann-Whitney U-test. The Pearson equation was used for correlation analysis. In all tests, p values less than 0.05 were considered significant.

RESULTS
Characteristics of burn patients included in the present study are presented in Table 1. All patients had been exposed to flames, one patient also presented with inhalation injury. A diagnosis of systemic inflammatory response syndrome (SIRS) was made in all patients.

The temporal changes of sICAM-1, sVCAM-1 and sE-selectin levels after thermal injury are shown in Fig. 1.

Among the sCAMs measured, sICAM-1 and sE-selectin levels showed the greatest increase in patients with burns. The level of sICAM-1 increased (137%) with maximum values six days after thermal injury. In contrast, sE-selectin increased (169%) from baseline very soon with maximum values at two days after thermal injury. After 30 days, a complete normalization was observed. In comparison with sICAM-1 and sE-selectin, sVCAM-1 showed only a slight increase. Both CRP and fibrinogen levels were maximally increased six days following thermal injury.

The results of measurements of CRP and fibrinogen in the plasma of patients with burns at different times after the burn injury are shown in Table 2. Mean CRP and fibrinogen levels significantly rose in patients, reaching a maximum on day six after thermal injury.

Correlations between sCAMs, CRP and fibrinogen are shown in Table 3. sICAM-1 and sVCAM-1 are significantly correlated with both CRP and fibrinogen (p < 0.001). A weak correlation is found between sE-selectin and both CRP and fibrinogen (p < 0.05).

The temporal changes of parameters of lipid metabolism in patients with thermal injury are shown in Fig. 2.

There was a decrease in levels of total cholesterol, LDL cholesterol, HDL cholesterol and nonHDL cholesterol by 12.2%, 17.5%, 28%, 7.4% respectively. In contrast, plasma triglyceride levels increased by 14.5% four days after the burn.

Table 4 shows the correlations between the levels of soluble CAMs and parameters of lipid metabolism.

There were negative correlations between total cholesterol, LDL cholesterol, HDL cholesterol and the levels of soluble adhesion molecules. However, no association between adhesion molecules and both triglycerides and nonHDL cholesterol was observed.

Table 1: Characteristics of patients with burns

<table>
<thead>
<tr>
<th>No. of patient</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>TBSA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>13</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>19</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>29</td>
<td>45</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>69</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>24</td>
<td>17</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>27</td>
<td>36</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>30</td>
<td>34</td>
</tr>
</tbody>
</table>

M = male, F = female, TBSA = Total burn surface area

Table 2: Time sequence of levels of CRP and fibrinogen (* p < 0.02 compared with day 0, # p < 0.04 compared with day 0)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time after injury</th>
</tr>
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<tbody>
<tr>
<td>CRP (mg/dl)</td>
<td>0     2     4     6    8     18    30 (days)</td>
</tr>
<tr>
<td></td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>fibrinogen (mg/dl)</td>
<td>226 ± 36</td>
</tr>
</tbody>
</table>

Table 3: Linear correlations between soluble cell adhesion molecules (sICAM-1, sVCAM-1, sE-selectin) and CRP and fibrinogen (r = correlation coefficient according to PEARSON)

<table>
<thead>
<tr>
<th></th>
<th>sICAM-1</th>
<th>sVCAM-1</th>
<th>sE-selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>r = 0.78</td>
<td>r = 0.54</td>
<td>r = 0.36</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>fibrinogen</td>
<td>r = 0.76</td>
<td>r = 0.55</td>
<td>r = 0.41</td>
</tr>
<tr>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>p &lt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>
In the present study, levels of sCAMs following burn injury and the correlation with other inflammatory parameters and parameters of lipid metabolism were evaluated. The results of this study demonstrate an increase of sE-selectin, sICAM-1 and sVCAM-1. There was a strong positive correlation between sICAM-1 and the routine markers of inflammation, CRP and fibrinogen.

In the current study, a characteristic time sequence of changes of sCAM levels was found. We showed that plasma sICAM-1 concentrations were increased during the first 30 days post-burn, with maximum level (2.3-fold increase) on day six. A moderate rise was found in plasma sVCAM-1, with maximum level (1.6-fold increase) also on day six. In contrast, maximum levels of sE-selectin were reached two days after thermal injury (2.7-fold increase). The early increase of sE-selectin may explain the very weak correlation of this CAM with CRP and fibrinogen. In accordance with this observation, upregulation of E-selectin expression on the surface of endothelial cells is an early step in inflammation. In contrast to other CAMs, E-selectin is expressed only on activated endothelial cells. Soluble E-selectin levels reflect its surface expression on vascular endothelial cells (1, 2, 8). Thus, the monitoring of sE-selectin levels could be particularly useful in the follow-up of patients with severe burns.

The serum levels of total cholesterol, LDL cholesterol, HDL cholesterol and nonHDL cholesterol decreased four days after the burn episode. The average reduction was 12.2%, 17.5%, 28.0% and 7.4%, respectively. In contrast, serum triglyceride levels increased 14.5%, four days after the burn. These data are in agreement with other observations regarding altered lipoprotein levels in patients with burns (17, 18). Moreover, the measurement of cholesterol and triglycerides was considered as clinically relevant to assess the morbidity of burn patients and thereby to estimate the patients outcome (19). The results in this study show a strong inverse relationship between sCAMs (sICAM-1, sVCAM-1, sE-selectin) and cholesterol-rich lipoproteins (total cholesterol, LDL cholesterol, HDL cholesterol).

There is proof that the elevation in sCAM concentration and inflammatory parameters that occurs during the burns is largely responsible for the rapid and marked decrease in cholesterol, LDL cholesterol and HDL cholesterol, mediated through effects of inflammatory mediators (ie cytokines) on lipoprotein metabolism (20, 21). Secretory phospholipase A2 (sPLA2), an acute-phase protein, may play a key role in the decrease of HDL cholesterol concentrations (22). Inhibition of lipoprotein lipase under the conditions of burn injury could lead to an impaired catabolism of triglyceride-rich lipoproteins and increase of the serum triglyceride level (18).

**CONCLUSION**

Further investigations involving a larger number of patients with burns should help to clarify the extent to which
measured parameters, especially the temporal changes of sCAMs, could be relevant to assess the morbidity of patients with thermal injury.

REFERENCES


