Soluble Cell Adhesion Molecules and Parameters of Lipoprotein Metabolism in Patients with Severe Burns

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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

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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 período de 30 días, fueron medidas a fin de determinar la participación de estos factores en la*

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patofisiología de las quemaduras severas. Los niveles séricos de sICAM-1, sVCAM-1 y sE-selectina fueron determinados mediante ELISA. Además, se midieron el colesterol total, el colesterol de lipoproteína de alta densidad (HDL col), el colesterol de lipoproteína de baja densidad (LDL col), y los triglicéridos.

Resultados: *Los niveles de sangre de sICAM-1, sVCAM-1, CRP y fibrinógeno aumentaron a valores máximos, seis días después del daño térmico. Los niveles de sICAM-1, sVCAM-1 y sE-selectina tuvieron una correlación significativa tanto con la CRP como con los niveles de fibrinógeno. El colesterol total de plasma, el colesterol HDL y el colesterol LDL disminuyeron a valores mínimos cuatro días después del daño térmico. Además, se observó un aumento en los niveles de triglicéridos.*

Conclusión: *La respuesta inflamatoria observada de las moléculas de adhesión celular soluble puede ser útil para monitorear la activación endotelial inmediatamente luego del daño térmico. Estudios ulteriores que comprendan un gran número de pacientes con quemaduras deben ayudar a aclarar hasta que punto los parámetros medidos, especialmente los cambios temporales de sCAMs, pudieran ser relevantes a la hora de evaluar la morbilidad de los pacientes con heridas térmicas.*

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INTRODUCTION

Activation of endothelial cells with increased expression and release of leukocyte adhesion molecules and changes of lipoprotein 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 Daignostics Systems – Reflotron 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.

Table 1: Characteristics of patients with burns

No. of patient	Gender	Age (yr)	TBSA (%)
1	F	13	24
2	M	19	15
3	M	29	45
4	M	69	31
5	F	24	17
6	M	27	36
7	M	30	34

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

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

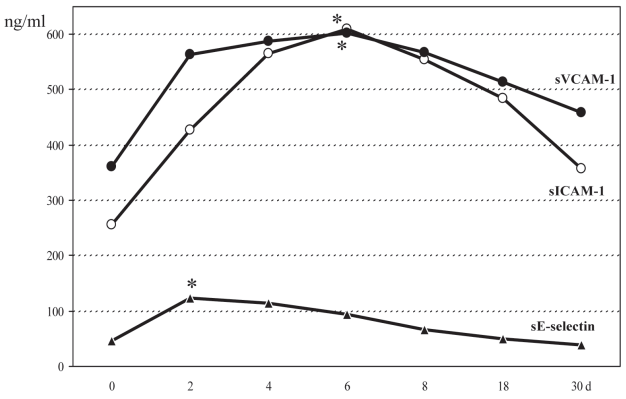

Fig 1: Time sequence of soluble adhesion molecules (sICAM-1, sVCAM-1, sE-Selectin) in burn patients (* $p < 0.001$ compared with day 0).

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 ($p < 0.05$).

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)

	sICAM-1	sVCAM-1	sE-selectin
CRP	r = 0.78 $p < 0.001$	r = 0.54 $p < 0.01$	r = 0.36 $p < 0.05$
fibrinogen	r = 0.76 $p < 0.001$	r = 0.55 $p < 0.01$	r = 0.41 $p < 0.05$

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

Parameters	Time after injury						
	0	2	4	6	8	18	30 (days)
CRP (mg/dl)	1.2 ± 0.7	14 ± 4.1	20 ± 7.8	21 ± 9.3*	14 ± 8.2	12 ± 5.2	7.8 ± 4.7
fibrinogen (mg/ dl)	226 ± 36	390 ± 34	536 ± 67	550 ± 54#	517 ± 24	460 ± 16	406 ± 37

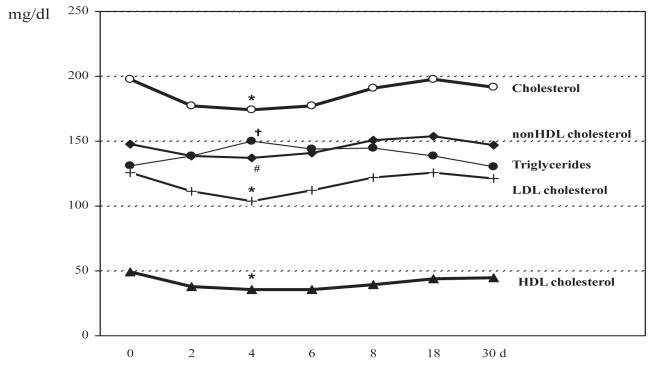

Fig. 2: Time sequence of levels of cholesterol, LDL cholesterol, HDL cholesterol, nonHDL cholesterol and triglycerides in burn patients (* $p <$ 0.001 compared with day 0, # $p <$ 0.04 compared with day 0, † < 0.02 compared with day 0).

Table 4: Linear correlations between soluble cell adhesion molecules (sICAM-1, sVCAM-1, sE-selectin) and cholesterol, HDL cholesterol, LDL cholesterol nonHDL cholesterol, triglycerides (r = correlation coefficient according to PEARSON, p = probability of error of the significance)

	sICAM-1	sVCAM-1	sE-selectin
Cholesterol	r = - 0.53 $p < 0.004$	r = - 0.51 $p < 0.006$	r = - 0.45 $p < 0.017$
LDL cholesterol	r = - 0.50 $p < 0.006$	r = - 0.45 $p < 0.021$	r = - 0.40 $p < 0.035$
HDL cholesterol	r = - 0.46 $p < 0.014$	r = - 0.46 $p < 0.014$	r = - 0.50 $p < 0.007$
nonHDL cholesterol	n s	n s	n s
Triglycerides	n s	n s	n s

(r = correlation coefficient according to PEARSON, p = probability of error of the significance)

DISCUSSION

Although animal models and clinical studies indicate that leukocyte – endothelial interactions and chemotactic cytokines are of importance in a number of pathophysiological events including inflammation (12–14), data on leukocyte adhesion molecules to determine their involvement in the pathophysiology of burns in humans are rare. Blood levels of E-selectin, ICAM-1 and VCAM-1 are increased in patients with respiratory disorder and systemic inflammatory response syndrome (SIRS) patients with respiratory disorder (10, 15). Increased levels of all these sCAMs in a variety of inflammatory diseases are well established and in many cases may be useful in predicting clinical outcome. Elevated plasma levels of sICAM-1 during the first 20 days post-burn were found.

Furthermore, soluble cell adhesion molecule concentration seem to be increased during septic shock and multiple organ dysfunction syndrome (10, 16).

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-selektin) 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.

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