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Dynamic changes of matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 after burn injury

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ABSTRACT

Purpose: Severe burn is a life-threatening condition. Many trials discuss the role of matrix metalloproteinases and tissue inhibitor of metalloproteinases in diseases generating systemic inflammatory response syndrome, and in some, their prognostic importance has been established. We aimed to describe the time courses of the aforementioned system and to evaluate the difference between survivors and nonsurvivors in burns.
Materials: Thirty-one patients were enrolled. Blood samples were collected on admission and on the 5 consecutive days. Circulating matrix metalloproteinase 9 (MMP-9) and tissue inhibitor of metalloproteinase 1 (TIMP-1) have been measured. Healthy individuals were invited as controls.
Results: Tissue inhibitor of metalloproteinase 1 increased in the burn group ($P < .001$) by day 2 and remained elevated thereafter. Plasma MMP-9 and MMP-9/TIMP-1 were already elevated on admission ($P < .001$) and decreased in tendency thereafter. In burned patients, significantly lower MMP-9 were noted on days 4 to 6 as MMP-9/TIMP-1 were also lower on days 3 to 6 ($P < .01$) compared with controls. We experienced difference regarding survival on days 5 and 6 by TIMP-1 ($P < .05$).
Conclusions: Our research is the first follow-up study elucidating the dynamic changes of MMP-9–TIMP-1 system in severe burns. Alteration of MMP-9–TIMP-1 balance might influence systemic inflammatory response and related mortality. Matrix metalloproteinase 9 might be a good injury marker in burns after an extensive trial.

1. Introduction

Matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) has a key role in the wound healing process and in remodeling [1-7]. Wound extracellular matrix is a key regulator of cell adhesion, migration, proliferation, and differentiation during cutaneous repair. The amount and structure of normal wound extracellular matrix are determined by a dynamic balance among overall matrix synthesis, deposition, and degradation. Matrix metalloproteinases are 1 family of structurally related enzymes that have the collective ability to degrade nearly all extracellular matrix components. The MMPs are broadly categorized into collagenases, gelatinases, stromelysins, and membrane-type MMPs by their substrate specificity [5].

Severe burns generally associated with systemic inflammatory response syndrome (SIRS) with attributed alteration of proinflammatory and anti-inflammatory cytokines in the early postinsult period [8,9]. Cytokines and growth factors can influence the expression of MMPs and TIMPs significantly [10], and therefore, the balance of MMP-TIMP system could be altered during the early postinjury period. Matrix metalloproteinases are able to modulate the inflammatory response; they are responsible for migration and extravasation of leukocytes mainly due to degradation of basal membrane [11], but according to recent evidence, they are also considered as signaling proteases. Tissue inhibitor of metalloproteinases can activate granulocytes and are able to protect inflammatory cells from apoptosis [12].

Matrix metalloproteinases and TIMPs have been evaluated extensively in sepsis [13-16], which is a feared and commonly fatal complication in burned patients. The prognostic importance of tissue inhibitor of metalloproteinase 1 (TIMP-1) has been proven, and the time course has been recently described in severe septic patients [15,16]. It has been proven in an animal burn model that matrix metalloproteinase 9 (MMP-9) enzyme activity is significantly increased after thermal injury and the basal lamina damage is reversed by inhibition of MMP-9 by doxycycline [17]. Matrix metalloproteinase 9 disintegrates the endothelial basal lamina of the blood brain barrier so it can be responsible for increased permeability and it may play role in the formation of the observed cerebral edema in peripheral thermal injury [18].

In human studies, the increase in MMP-9 levels was detected in the edema fluid on day 4 [2]. In a meta-analysis of 3 studies, 1.4-fold

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increase of serum TIMP-1 levels was found after burn injury [19]. Patients with extended hypertrophic scars after burn trauma presented a significantly higher TIMP-1 concentration in their sera than the other patients. There was no significant difference in the relative expression of messenger RNA of MMP-9 in scar tissue and keloids in comparison to nonscarred skin tissue [6]. In the study of Dasu et al [20], serum levels of MMPs and their tissue inhibitors were studied in burn patients over time. Matrix metalloproteinase 9 levels showed significant increase on day 21. Tissue inhibitor of metalloproteinase 1 levels showed a significant increase from day 3 after burn injury [6,20]. In another study [21], serum levels of MMP-9 showed a significant elevation only between days 3 and 14 in patients with burn wounds. Serum TIMP-1 levels were elevated from day 1 to 6 months after injury. The elevated systemic TIMP-1 concentration was thought to be responsible for pathologic scar formation [21]. Nowadays, this enzyme system is implicated as a new class of biomarkers with different expression pattern in many pathologic conditions. No data are available in severely burned patients on the time course of MMP-TIMP system in the early postburn period. There have not been investigations into the difference between survivors and nonsurvivors. The aim of our study was to provide these important and missing data to facilitate better understanding of the underlying process in burn injury.

2. Materials and methods

2.1. Subjects and study design

The study protocol was completed in accordance with the ethical guidelines of the 2003 Declaration of Helsinki. After receiving permission from the local ethics committee (4282.316-2216/KK15/2011), this descriptive study was performed using the remaining blood samples of patients who participated in our previous studies. Only the blood samples of patients taken between March 2005 and May 2010 were investigated. Ten healthy, age- and sex-matched individuals served as control. The patients were treated in uniform way, and they received no study medications. Inclusion and exclusion criteria were similar as in the original studies. Inclusion criteria were burn injury affecting more than 15% of the body surface and admission to our ward within 6 hours after injury. Exclusion criteria were electrical injury, presence of any obvious bacterial infection on admission, extreme burn severity (>80% total burnt surface area), previously documented chronic left heart or renal insufficiency, younger than 18 years, or documented previous medication affecting the inflammatory response of the body to burns (eg, chronic use of corticosteroid medication) and known malignant disease. Burned patients did not receive medications, which is considered affecting the inflammatory response—such as corticosteroids—during the trial. The fluid therapy of our patients was guided by invasive transcardiopulmonary hemodynamic monitoring (PiCCO; Pulsion Medical Systems, Munich, Germany) [22-24]. Excision and grafting were started within 72 hours, and enteral feeding was commenced on the first day after injury when hemodynamic stability was reached. Twenty percent to 30% burned surface was excised and grafted in 1 sitting. Operations were repeated every 3 to 4 days. In case of suspected long-term ventilation, tracheostomy was performed before the first grafting to avoid complications due to coagulopathy. If inhalation injury was suspected (facial burn, soot in the throat, and chest x-ray), bronchoscopy was carried out for verification. Blood samples were taken on admission (day 1) and on the 5 consecutive days (days 2-6) at 7 AM before operations or painful dressing changes. Six-day study interval has been chosen because we had presumed that a 6-day period would open a wide time window that could be enough for detecting both the ascending and descending inflammatory responses.

2.2. MMP-TIMP assays

Plasma was isolated from heparin anticoagulated blood samples by low speed centrifugation at 4°C, and stored at −80°C until analyzed in a single batch. Matrix metalloproteinase 9 and TIMP-1 were determined by the quantitative sandwich enzyme-linked immunosorbent assay techniques according to the manufacturer's instructions (R&D Systems, Inc, Minneapolis, MN). In comparison with standard MMP and TIMP curves, the concentrations of MMP and TIMP in plasma were determined spectrophotometrically (Multiskan Ascent microplate photometer, Type: 354; Thermo Electron Corporation, Waltham, MA) by reading the absorbance at 450 nm and were expressed as entire amounts in the plasma (nanograms per milliliter).

2.3. Statistical analysis

SPSS software, version 21.0 (IBM Corporation, Armonk, NY) was used for statistical analysis. Data were expressed as median and interquartile range (IQR [standard 25th-75th percentile and 5th and 95th confidence interval]) because their distribution was not normal by Kolmogorov-Smirnov test. Kruskal-Wallis test was used for intergroup analysis, and survivors to nonsurvivors and patients to healthy controls were compared with Mann-Whitney U test. Jonckheere-Terpstra test was used to detect significant trends. Correlations were analyzed with Spearman test. Values of $P < .05$ were considered significant.

3. Results

Thirty-one patients were involved in the study. Fourteen patients survived; 17 died. Demographic and clinical data are summarized in Table. We realized significant difference regarding age and extent of burn between survivors and nonsurvivors. By burns in general, the most frequent complications are sepsis and respiratory, cardiac, and renal failure. However, the leading causes of death during the trial period were cardiac (67%) and respiratory failure (33%), and we experienced no septic complications. No correlations were detected between enzyme levels and common severity scores (Multiple Organ Dysfunction Score, Sequential Organ Failure Assessment, and Simplified Acute Physiology Score II) on admission, but Abbreviated Burns Severity Index (ABSI) positively correlated with admission MMP-9/TIMP-1 ratios ($r = 0.454$; $P = .01$).

Table
Demographic and characteristic data of our study group

	All patients (n = 31)	Survivors (n = 14)	Nonsurvivors (n = 17)	P
Age (y)	51 (37-71)	44 (30-56)	58 (46-74)	<.05
Burned surface area (%)	30 (20-50)	24 (18-33)	40 (30-55)	<.01
Deep burn injury (%)	27 (18-35)	20 (15-25)	30 (27-40)	NS
Inhalation injury (n)	9	4	5	NS
Flame burn injury (n)	11	7	4	NS
Blast burn injury (n)	6	4	2	NS
Scald burn injury (n)	5	3	2	NS
SOFA score	7 (5-9)	7 (5-10)	7 (5-8)	NS
MODS	5 (3-6)	5 (4-7)	6 (3-6)	NS
SAPS II	31 (24-43)	27 (24-40)	34 (27-45)	NS
ABSI	8 (7-9)	8 (6-8)	8 (7-10)	NS
Renal failure (n)	10	3	7	NS
Hemodialysis (n)	10	3	7	NS
ICU stay (d)	10 (7-20)	10 (7-21)	10 (7-22)	NS

NS indicates nonsignificant; SOFA, Sequential Organ Failure Assessment; MODS, Multiple Organ Dysfunction Score; SAPS II, Simplified Acute Physiology Score II; ICU, intensive care unit.

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