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BRIEF REPORT

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Plasma oxidative parameters and mortality in patients with severe burn injury

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Abstract *Objective:* To determine xanthine oxidase and superoxide dismutase activities, lipid peroxidation, protein carbonylation, and total radical-trapping antioxidant parameter in survivors and nonsurvivors patients with severe burn injury. *Design and setting:* Prospective, comparative observational study in an intensive care unit, burn division, in a trauma hospital. *Patients:* Twenty-five consecutive patients who met the established criteria for severe burn injury (total burn surface area of more than 30%). *Measurements and results:* Plasma thiobarbituric acid reactive species and protein carbonyls levels were significantly higher in nonsurvivors than in survivors at 0 and 6 h. Elevated xanthine oxidase activity at 0 h was associated with adverse outcome after burn injury. In contrast, plasma superoxide dismutase activity and total radical-trapping antioxidant parameter did not differ significantly between nonsurvivors and survivors at any time point. *Conclusions:* For the first time we demonstrate the value of oxidative parameters, namely thiobarbituric acid reactive species, protein carbonyls, and xanthine oxidase activity, in identifying burn patients with a poor prognosis. Whether these parameters are merely markers of clinical course, or whether they signal specific deleterious effects of oxidative stress during the burn injury remains to be elucidated.

Keywords Burn · Oxidative stress · Superoxide dismutase · Lipid peroxidation · Protein carbonyl · Xanthine oxidase

Introduction

Major burn (total burn surface area, TBSA >30%) is associated with marked mortality. There is increasing evidence that oxidative stress has an important role in the development of multiple organ failure after major burn injury [1, 2, 3, 4]. The contribution of oxidants to postburn multiple organ dysfunction has been reported by several studies that show increased lipid peroxidation and decreased antioxidant activity after burn. Additional support for the role of oxidant-mediated injury after burn trauma has been comes from studies demonstrating the protective effects of antioxidant therapies in burn trauma [3, 4].

These findings suggest the possibility of developing new therapeutic approaches and new plasma markers of severity in the clinical setting. However, the association of oxidative stress parameters and postburn prognosis has not been studied extensively in severely burned patients. Therefore the present study aimed at determining plasma oxidative parameters in severely burned patients and its relationship to postburn mortality.

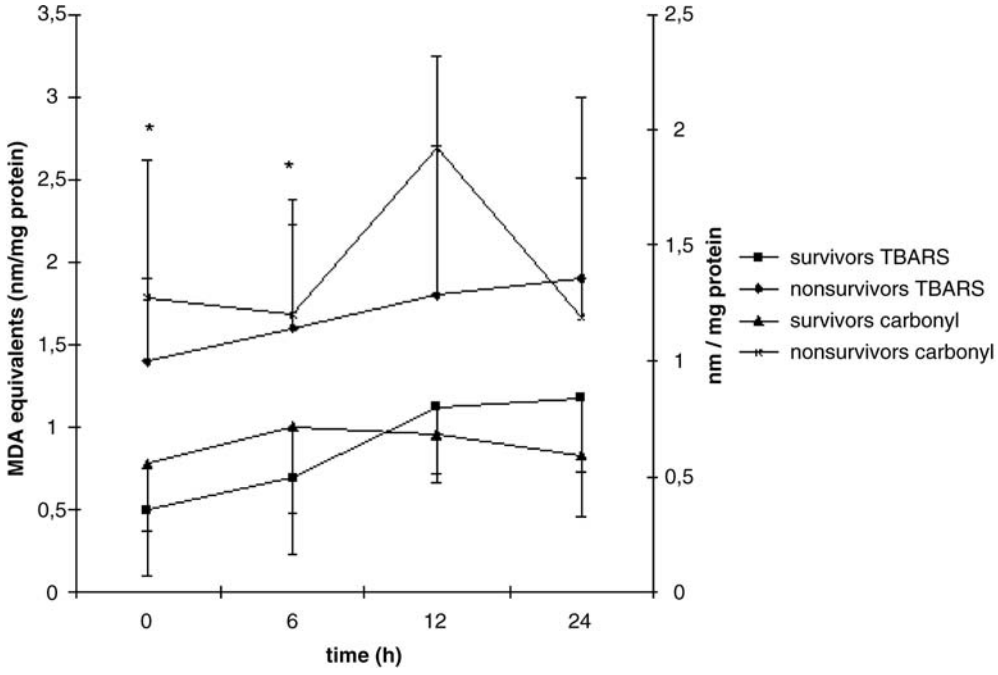

Fig. 1 Plasma concentrations of thiobarbituric acid reactive species and protein carbonyls in severe burn patients. Blood samples were collected at times 0 (up to 6 h from accident) and 6, 12, and 24 h after hospital admission. Plasma thiobarbituric acid reactive species and protein carbonyls were measured as described in the text comparing survivors (n=10) and nonsurvivors (n=15). Results are expressed as means ±SD. *p<0.05 vs. survivors

Material and methods

Study population

Patients admitted to the Intensive Care Unit at the Hospital de Pronto Socorro with a TBSA greater than 30% caused by flame or scalds within 6 h of the accident were considered eligible for the study. Exclusion criteria were: electrical burn lesions, age less than 16 years, pregnancy, decompensated heart failure, uremia, neoplasia, autoimmune disorders, recent cardiovascular events, and use of steroids. The population comprised 25 patients: 10 survivors (age 31.4±13 years) and 15 nonsurvivors (age 36.4±15 years). The distribution by age and gender did not differ significantly between the groups, nor did the period from injury to study entry (3.9 h in survivors, 3.8 h in nonsurvivors). The protocol was approved by the ethics and research committees at Hospital de Pronto Socorro and Hospital de Clínicas, Porto Alegre. All patients gave their informed consent prior to their inclusion in the study. Acute Physiology and Chronic Health Evaluation (APACHE) II scores were obtained from all cases at entry. The development of multiple organ dysfunction syndrome was recorded daily until discharge. The management of patients followed the institution's standard protocol, without interference from the investigators.

Blood sampling

Immediately after arrival at the hospital (time 0), and 6, 12, and 24 h thereafter blood samples were collected from patients. The material was immediately centrifuged and stored at −70°C until analyses.

Oxidative stress parameters

As an index of lipid peroxidation we used the formation of thiobarbituric acid reactive species (TBARS) [5]. Briefly, the samples were mixed with thiobarbituric acid 0.67%. TBARS were determined by the absorbance at 535 nm and expressed as malone dialdehyde equivalents (nm/mg protein). The oxidative damage to proteins was assessed by determination of carbonyl groups based on the reaction with dinitrophenylhydrazine as previously described [5]. Briefly, proteins were precipitated, dissolved in dinitrophenylhydrazine and the absorbance read at 370 nm. Values are expressed as nm/mg protein. Total radical-trapping antioxidant parameter (TRAP) was assayed as previously described [6]. Briefly, the reaction was initiated by injecting luminol and a free radical generator. The addition of plasma (150 μg protein) decreases the luminescence proportional to the sample concentration of nonenzymatic antioxidants. Total superoxide dismutase (SOD) activity was assayed by measuring the inhibition of adrenaline auto-oxidation, as previously described [7]. Activity of xanthine oxidase (XO) was assayed using oxidation of pterin to isoxanthopterine [8].

Statistical analysis

Data are expressed as mean ±standard deviation for variables with normal distribution. For repeated measurements a two-factorial analysis of variance was performed. Comparison between means was carried out using the Newman-Keuls test. Multivariate analyses were performed to control for potential confounding factors: age, APACHE II, TBSA, and inhalation injury. Results are reported as unadjusted means. Pearson's correlation test was used to study associations between marker levels and other variables. Differences with p values below 0.05 were considered significant.

Results

As expected, TBSA (39.5±11.7 vs. 56.6±18, p=0.01), APACHE II scores (9.6±4.9 vs. 15.7±6.1, p=0.01), and multiple organ dysfunction syndrome (0.46±0.8 vs. 2.7±1.6, p=0.01) differed significantly between survivors and nonsurvivors. Survivors and nonsurvivors also differed in terms of the incidence of inhalation injury (40%

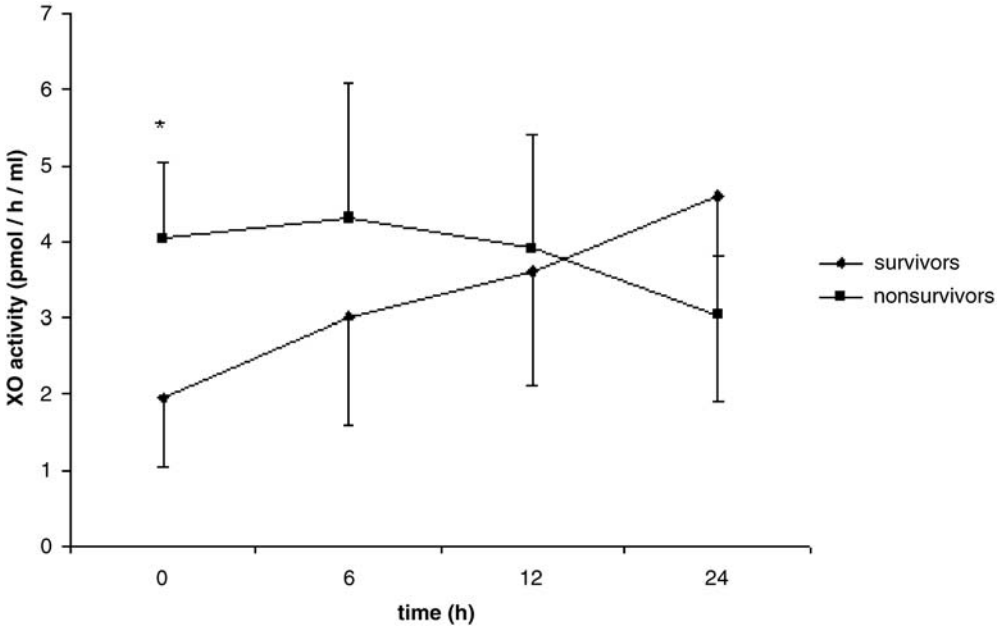

Fig. 2 Plasma xanthine oxidase activity in severely burn patients. Blood samples were collected at 0 (up to 6 h from accident) and 6, 12, and 24 h after hospital admission. Plasma xanthine oxidase activity was measured as described in the text comparing survivors (n=10) and nonsurvivors (n=15). Results are expressed as means ±SD. *p<0.05 vs. survivors

vs. 86% respectively, p=0.05). TBARS and carbonyl levels seem to be an early marker of adverse outcome after burn injury. Both levels were significantly higher in nonsurvivors at 0 and 6 h (Fig. 1). In contrast, 12 and 24 h after hospital admission TBARS and carbonyls levels were not significantly higher in nonsurvivors than in survivors. The results did not change after adjustment for TBSA, age, inhalation injury, and APACHE II. In nonsurvivors with inhalation injury (n=13) there were a trend toward higher TBARS at 0 h (1.4±0.3) and 6 h (1.6±0.5) in comparison to survivors with inhalation injury (n=4; 0 h, 0.63±0.2; 6 h, 0.74±0.28). These were also true for protein carbonyls values (data not shown). In nonsurvivors with a TBSA between 30–45% (n=5) there were higher TBARS values at 0 h (1.3±0.3) than in survivors with comparable TBSA (n=8; 0.4±0.2). Similar results are observed when analyzing those with TBSA greater than 45 (data not shown). These findings reinforce the results from multivariate analyses. TBSA, inhalation injury, and oxidative parameters emerged as independent prognostic parameters.

Elevated XO activity at 0 h was associated with adverse outcome after burn injury (Fig. 2). In contrast, 6, 12, and 24 h after hospital admission XO activity was not significantly higher in nonsurvivors than in survivors. The results did not change after adjustment for TBSA, age, inhalation injury, and APACHE II. Plasma SOD activity was not statistically different between nonsurvivors and survivors at any time point (data not shown). However, there was a trend toward higher SOD activity among nonsurvivors at time 0 (4.0±1.7 vs. 7.1±1.8, respectively p=0.07). There was a trend toward plasma TRAP values be less antioxidant in nonsurvivors than in survivors at all times tested (data not shown), indicating a consumption of nonenzymatic antioxidant defense. No significant correlation was found between APACHE II and levels of TBARS, protein carbonyls, and XO activity at time 0 (R^2=0.022, p=0.7). There was a significant correlation between TBSA and TBARS and protein carbonyls levels at most time points (data not shown). Finally, XO and SOD activity were not correlated with TBSA at any of the time points (data not shown).

Discussion

Several mediators seem to be critical during the organic response to burn injury. However, the role of these mediators as predictors of mortality in burn patients is only recently beginning to be explored [9]. The presence of oxidative damage to lipids during the course of burn injury is well documented. Bertin-Maghit et al. [10] demonstrated that TBARS levels rise during the first 5 days after burn injury. Pintaudi et al. [11] demonstrated markedly increased in plasma levels of malone dialdehyde after burn injury. There are no studies in the literature that demonstrate the presence of protein carbonyls in humans with severe burn injury or the value of an early increase in TBARS and protein carbonyls levels as markers of mortality as we demonstrate in Fig. 1. In our sample plasma TBARS values higher than 0.6 nm/mg protein at times 0 and 6 predicted mortality with 100% sensitivity and 88% specificity and with 100% sensitivity and 75% specificity, respectively. In addition, plasma carbonyls values higher than 1.0 nm/mg protein at times 0 and 6 predicted mortality with 85% sensitivity and 70% speci-

ficity and with 90% sensitivity and 68% specificity, respectively.

The appearance of XO activity in the human plasma has been confirmed in patients with sepsis syndrome [12]. We have found no studies on XO activity during burn injury in humans. We show for the first time that XO activation is increased in patients with severe burn injury, and that this predicts outcome at time 0. In our sample plasma XO activity higher than 3.0 pmol/ml per hour at time 0 predicted mortality with 90% sensitivity and 100% specificity.

Saitoh et al. [13] measured three types of SOD in plasma of burned patients and demonstrated that any plasma SOD isoenzyme concentration measured after burns is beyond the normal range. In this study plasma extracellular SOD was significantly related to existence of inhalation injury, TBSA, and age [13]. In contrast, we observed only a trend toward a higher SOD activity among nonsurvivors at time 0.

Demling et al. [14] demonstrated that lower systemic antioxidant capacity level and increased lipid peroxidation were correlated with a fatal outcome after a burn in an animal model. In contrast, Farriol et al. [15] reported no correlation between total antioxidant capacity, percentage of burned surface, and clinical course in 32 burned patients. We demonstrated a tendency to lower antioxidant potential in nonsurvivors burned patients at all times tested.

Our results should be interpreted in the context of limitations and characteristics of the study. First, due to technical limitations inhalation injury was diagnosed based on clinical parameters only; this may have led to overdiagnosis of inhalation causes. Second, this report describes a small population of patients; thus the changes in oxidative parameters, especially the subgroups analyses and the values for sensitivity and specificity, show differences that must be confirmed by studies with larger numbers of patients.

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