Hypothesis: Most investigators have reported high levels of endothelin (ET)-1 in patients with thermal injury. We attempted to examine the hypothesis that ET-1 levels increase in patients with severe burn injury.

Patients and Methods: Plasma from 28 adult subjects, 14 of whom had thermal injuries with a median (range) percentage of total burn surface area of 22% (20%-76%), was assessed for ET-1 and tumor necrosis factor (TNF) α. Samples from closely age-matched patients were obtained on admission (day 1) and 24 hours postinjury (day 2). Samples were obtained before blood transfusion or surgical treatment occurred. Enzyme immunoassay techniques suitable for the measurements of the cytokines were used.

Results: Median (range) of TNF-α was higher in patients (day 1, 10.0 ng/L [1.2-35.0 ng/L]; day 2, 12.0 ng/L [0.4-39.0 ng/L]) than controls (0.8 ng/L [0.3-3.2 ng/L]) (P<.005) while ET-1 levels remained significantly unchanged in patients (mean [SD], day 1, 183.0 [42.2] ng/L; day 2, 204.7 [41.7] ng/L) compared with controls (170.0 [59.8] ng/L) (P>.05).

Conclusions: We observed no significantly raised levels of ET-1 in patients with thermal injury within 24 hours after burn injury. We found no significant correlation between the plasma levels of TNF-α and ET-1. Endothelin-1 levels did not seem to reflect severity of illness. The actual evaluation of ET-1 release in patients with thermal injury could enhance the pathophysiological study of human thermal injury.

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Endothelin-1 Levels in Severe Burn Injuries

Gracey N. Onuoha, PhD; E. Kaya Alpar, FRCS; John Gowar, FRCS

THERMAL INJURY is generally associated with immunologic dysfunction resulting from alterations in cytokine responses and has been linked to the release of proinflammatory and anti-inflammatory mediators. The former group of mediators includes tumor necrosis factor (TNF)-α,1 while the latter includes endothelin (ET)-1.2 Immunological alterations of ET-1 increase the risk of development of infection, originating either from wounds or remote sites, and these can further increase the mediator release, alter hemodynamic status, and result in increased morbidity and mortality. Endothelin-1 is a powerful vasoconstrictor, a peptide with 21 amino acids, and is produced by ischemic or injured endothelial cells. Endothelin-1 affects pulmonary, hepatic, cardiac, and renal function.3 It also causes monocyte production of TNF-α4 and a substance that causes neutrophil production of superoxide.4 While plasma levels of ET-1 are one thousandth that of tissue levels of ET-1, they do seem to reflect ischemic or injury events in the macrovasculature.

Endothelin-1 is a candidate mediator for an important role in the genesis of the systemic response to burn injury and might also have a role in immune and inflammatory dysfunction. Endothelin-1 has been shown to increase after myocardial infarction,5 after perfusion that occurs after coronary thrombosis,6 in low-flow states,7 with pulmonary hypertension,8 in burns, and in sepsis.7 However, in our study there was no significantly raised level of ET-1 in these patients with thermal injuries. We also measured the release of TNF-α, one of the first cytokines shown to have diverse regulatory properties in immunity and inflammation.9 Systemic changes during inflammation such as increased acute-phase protein synthesis and fever probably represent action of this cytokine on the liver and hypothalamus. This cytokine acts in a paracrine fashion in the local environment to stimulate immune responses but also acts in an endocrinelike fashion on distant organs that participate in inflammatory responses. It increases in

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PATIENTS, METHODS, AND MATERIALS

PATIENTS

Fourteen adult patients (mean age, 52.6 years; lower and upper quartile ranges, 29-94 years; 10 men) with thermal injury were resuscitated with a modified Parkland formula to a urine output of about 30 mL per hour. These patients were compared with 14 healthy closely age-matched control subjects (mean age, 51.4 years; lower and upper quartile ranges, 24-86 years; 7 men). Mean (SD) percentage of total burn surface area (%TBSA) was 35.0 (23.1).

Ninety one percent of patients had burns on the upper extremities, including hand, neck, thorax, and upper back; 7 (50%) had burns on the lower extremities, including abdomen and lower back, some with mixed burns; and 10 (71%) of patients survived. Patient’s demographic details are given in Table 1. Plasma was drawn immediately on admission (day 1) and 24 hours after admission (day 2). Samples were obtained from subjects through a venipuncture and into EDTA aprotinin bottles in a protocol approved by the institutional review committee for University of Birmingham, Birmingham, England. Samples were obtained from patients before blood transfusion or surgical intervention. Immediately on arrival to the laboratory and within one-half hour after sampling, the venous blood samples were centrifuged for 15 minutes at 5000 rpm at 0°C. Aliquots of plasma were placed in polypropylene tubes stored at −80°C until the tests were performed. The Ethics Committee of University of Birmingham approved this study, and informed consent was obtained from all subjects or care givers.

LABORATORY ANALYSIS

Enzyme Immunoassay

The levels of ET-1 and TNF-α were measured using commercially available enzyme immunoassay kits (ET-1, EIA 6901 [host, rabbit] and TNF-α, ACCUCYTE; both from Peninsula Laboratories, Merseyside, England) as per instructions. The plasma analysis of ET-1 required extraction using separation-column buffer containing 200 μg of C-18 (catalogue number RIK-SEPCOLI; Peninsula Laboratories), equilibrated once with 1 mL of 100% acetonitrile. This was followed by washing 3 times with 3 mL of Buffer A (1% trifluoroacetic acid in 99% distilled water) (catalogue number BUF-A1; Peninsula Laboratories). The plasma was acidified with an equal amount of Buffer A to plasma and clarified by centrifugation at 10000 rpm for 20 minutes at 4°C, and the supernatant passed through the pretreated cartridges. After washing the column slowly 3 times with 3 mL of Buffer A, the peptide was then slowly eluted into a polypropylene tube, and the eluant evaporated to dryness in a centrifugal concentrator.

The dried extract was reconstituted with assay buffer prior to assay analysis. No extraction was required for TNF-α. During analysis, known amounts of ET-1 and TNF-α were incubated with their specific antibodies, and these samples subsequently were incubated with biotinylated-labeled peptides. After removing unbound biotinylated peptide by washing, streptavidin-conjugated horseradish peroxidase was added. After washing away excess streptavidin-conjugated horseradish peroxidase, tetramethyl benzidine dihydrochloride was allowed to react with bound horseradish peroxidase. All samples were performed in duplicates and absorbance read with a spectrophotometer at 450 nm for ET-1 and at 492 nm for TNF-α. These numbers were then fitted to the standard curve to derive numeric values.

Endothelin-1

The lowest detectable concentration of ET-1 was 0.04 to 0.06 ng/mL of the plasma sample (tracer, biotinylated ET-1). There was 100% cross reactivity with ET-1 (human); 7%, ET-2 (human, canine); 0.05%, ET-3 (human, rat, porcine, rabbit); 17%, big ET-1 (human); and 0%, big ET-22-38 (human), vasoactive intestinal peptide, and brain natriuretic peptide-32 (human). Intra-assay and interassay coefficients of variation were 5% and 14%, respectively.

Human TNF-α

The lowest detectable concentration of TNF-α was 0.195 ng/mL of the plasma sample. The tracer was biotinylated TNF-α. Intra-assay and interassay variations for human TNF-α were 6.5% and 11.7%, respectively.

Statistics

Results are expressed as median (lower and upper quartile range) in nanograms of the peptide per liter of plasma sample. Nonparametric statistic (Wilcoxon rank sum test) was used. Mean and SD were calculated to describe continuous variables. The degree of correlation between peptide scores and %TBSA was calculated using a Spearman rank correlation coefficient. P<.05 was considered significant, statistically.

RESULTS

This study focused on those patients with an early tissue injury during the time before burn wound sepsis or systemic sepsis occurs. No patient had cardiac disease, experienced shock, or had multiple system organ failure. None of the patients received anticoagulants, steroids, blood transfusion, or surgical treatment within the time of blood sampling. As a criterion for the work, only adult patients (>16 years) were accepted for the study to complement the age of the control subjects.

In the control group, the median (lower-upper quartile range) values were 198 ng/L (72-229 ng/L) for ET-1 and 0.8 ng/L (0.25-3.20 ng/L) for TNF-α. There was no significantly raised level of ET-1 between patients at admission (198 ng/L [117-229 ng/L]) or 24 hours postin-
jew (207 ng/L [117-270 ng/L]) compared with controls (P>.05). The TNF-α levels were significantly raised in patients (day 1, 10.0 ng/L [1.2-35.0 ng/L]; day 2, 12.0 ng/L [0.4-39.0 ng/L]) compared with controls (P<.005). The release of TNF-α in patients was at least 10 times higher than that in control subjects. A higher level of TNF-α was obtained at day 2 of our study; however, this variation did not correlate with any differences in %TBSA, ET-1 release, or presence of a pulmonary injury. Table 2 summarizes the results.

COMMENT

In this study the effect of tissue injury on circulating levels of ET-1 and TNF-α has been evaluated and compared in patients with thermal injury and age-matched healthy controls. Basal levels of ET-1 were not significantly changed while TNF-α levels increased in patients with thermal injury and in the concentrations maintained within 24 hours after thermal injury. Several cytokines, monokines, enzymes, and lipids have been identified that change dramatically in burn injury and may have a role in either the local or systemic response to thermal injury or wound healing.10,11 However, whether any single mediator or factor can be identified and changed to improve outcome remains uncertain. Endothelin-1 is a 21 amino acid peptide formed in the endothelial cells and produced by injured or ischemic vascular endothelium.3 While originally identified as a potent vasoconstrictor, it was shown to be a potent bronchoconstrictor, visceral smooth muscle contractior, and a secretagogue and growth factor for many cells.

At least 6 endothelins (big ET-1,12 ET-1,13 big ET-2,14 ET-2,15 big ET-3,16 and ET-317) have been identified in the human genome. Of those, only ET-1 has been studied thoroughly. Endothelin-1 is a bronchoconstrictor and pulmonary vasoconstrictor; has both negative and positive cardiac inotropic effects; stimulates hepatic Kupffer cells18 and hepatocellular glycogenesis,19 and inhibits bile flow.20 It is a renal vasoconstrictor,21 gut mucosal ulceragen, neuroendocrine stimulant,22 and leukocyte activator; it stimulates proliferation of smooth muscle cells23 and causes TNF-α release.24 Thus, ET-1 is a candidate mediator in the systemic response to burn injury and a potential stimulus to activation of the immune response and inflammatory mechanisms. Endothelin-1 is also known to cause monocyte production of transforming growth factor and basic fibroblast growth factor, suggesting that this neuropeptide may have a role in wound healing. However, the measurement of the specific isoform remains difficult.

The levels of ET-1 in patients with thermal injuries has been inconsistent in terms of its actual release or measured levels. While some reports suggest increased levels of the peptides in the peripheral blood samples of patients with burn injury,11 others observed reduced levels of the cytokine.25 In this study, using commercially available ET-1 kits, our results show insignificant differences in levels between patients and age-matched controls. Although many studies describe moderate elevation of plasma ET levels in patients with thermal injuries, including severe burns,11,26,27 it remains unclear if this elevation is that of the biologically active peptide ET-1 or of its precursor, big ET.

Endothelin-1 is a member of a family of peptides arising from preendothelial and proendothelial species, which after proteolysis yields 3 isoforms termed ET-1, ET-2, and ET-3. Big ET may be the main circulating form of ET in man.28 The report by Huribal et al26 for instance, indicates that the elevations of plasma ET levels in severe burns is primarily related to elevated circulating ET-1. Their study revealed the presence of ET of similar activity without any big ET activity, thus suggesting that thermal injuries might not be the main cause of raised circulating ET concentrations. Furthermore, it is not clear if such increases in patients is associated with elevated tissue ET levels. Most of these non–age-matching patient and control groups do not provide evidence of the stimulus for its release.27 Possible sources could include microvascular injury from the burn, underperfused tissues for local or systemic reasons, sepsis, increased ET-1 production by monocytes activated by injury, or decreased clearance of the peptide by local or hepatic metabolic mechanisms.

In some of these studies, there was no mention of the specificity or cross reactivity of the peptide to its different isoforms.11,25 and often the ET-1 was not extracted from the assay matrix.20,27 The extraction procedure not only allows for the concentration of the sample but also serves to separate peptides from potentially interfering substances. A measurement of ET-1 in plasma is difficult because of the presence of the 3 different isoforms and of precursors such as big ETs. Assays frequently measure both bioactive fragments and precursor, or cross react, between isoforms, and in some cases the cross reactivity could be as much as 100%.20 This suggests that the high levels reported in some results could be owing to the cross reactions of different isoforms other than ET-1 itself. Our assay has less than 20% cross reactivity between ET-1 and big ET-1. Therefore, al-

### Table 1. Patient Demographics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, No.</td>
<td>14</td>
</tr>
<tr>
<td>Age, y</td>
<td>52.6 ± 18.8</td>
</tr>
<tr>
<td>Sex, M</td>
<td>10</td>
</tr>
<tr>
<td>Mean ± SD, %TBSA</td>
<td>35 ± 23</td>
</tr>
<tr>
<td>Inhalation, No. (%)</td>
<td>8 (57)</td>
</tr>
<tr>
<td>Mortality, No. (%)</td>
<td>4.0 (28.6)</td>
</tr>
</tbody>
</table>

* %TBSA indicates percentage of total burn surface area. Of the injuries, 79% were accident oriented; 7%, assault; and 14%, self-inflicted.

### Table 2. Biochemical Data of 14 Patients and 14 Controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>At Admission</th>
<th>24 h Postinjury</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD, ET-1, ng/L</td>
<td>183 ± 42†</td>
<td>205 ± 42†</td>
<td>170 ± 60</td>
</tr>
<tr>
<td>Mean ± SD, TNF-α, ng/L</td>
<td>17 ± 16‡</td>
<td>18 ± 16‡</td>
<td>11 ± 1.0</td>
</tr>
</tbody>
</table>

* ET-1 indicates endothelin-1; TNF-α, tumor necrosis factor α. †P<.05 compared with controls. ‡P<.005.
though previous studies have indicated a modest elevation of circulating ET-1 levels in patients with thermal injuries, the failure to do so here could relate to methodological differences. Our findings are in agreement with the reports of Areta et al. 

We also measured the release of TNF-α. Significantly high TNF-α levels were observed in patients with thermal injury, and these levels were maintained within the time of the study. Tumor necrosis factor α is crucial in the initiation of humoral and cellular immune responses and stimulates systemic changes during inflammation, including synthesis of acute-phase proteins and the induction of fever. Tumor necrosis factor α was among the first monocyte-derived cytokines shown to have diverse regulatory properties in immunity and inflammation. It may also induce tissue destruction in chronic inflammatory disease. This monocyte causes physiological and pathological alteration in the host.

One of the biological effects of ET is its ability to cause the proliferation of smooth muscle cells, thus in these thermally injured patients, ET-1 may play an important role in the healing of wounds. The strong, characteristically long-lasting vasoconstrictor activity of ET-1 may also be important in the control of systemic blood pressure and/or local blood flow; hence, disturbance in the control of ET-1 production could affect wound healing in patients with thermal injuries.

An attempt has been made in this study to confirm the peripheral release levels of ET-1 in closely age-matched patients and controls. We observed no significant changes in ET-1 release in this nonseptic group of patients within 24 hours postinjury. The peripheral levels of ET-1 in patients with thermal injury have been inconsistent. The actual evaluation of ET-1 in patients with thermal wounds could enhance the pathophysiological study of human burns, opening doors to greater useful clinical information about flame injuries.

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REFERENCES


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