Neuroendocrine System Response Modulates Oxidative Cellular Damage in Burn Patients

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It is recognized that extensive thermal trauma introduces damage in multiple organs remote from the original burn wound and may lead to fatal multiple organ failures (Foster et al. 1996; Umetzu and DeKruyff 1997). The release of massive inflammatory mediators is associated with these failures (Cakir and Yegen 2004; Sener et al. 2005). A vast number of evidences demonstrate oxygen-derived free radicals and high-energy oxidants such as peroxynitrite accompany-

Oxygen-derived free radicals play important roles in pathophysiological processes in critically ill patients, but the data characterizing relationships between radicals and neuroendocrine system response are sparse. To search the cue to reduce the oxidative cellular damage from the point of view of neuroendocrine system response, we studied the indicators of neuroendocrine and inflammatory responses excreted in urine in 14 burn patients (42.3 ± 31.4 years old, and 32.3 ± 27.6% burn of total body surface area [%TBSA]) during the first seven days post burn. The daily mean amounts of urinary excretion of 8-hydroxy-2′-deoxy-guanosine (8-OHdG), a marker of oxidative cellular damage, were above the upper limit of the standard value during the studied period. The total amount of urinary excretion of 8-OHdG in the first day post burn correlated with burn severity indices: %TBSA ($r = 0.63$, $p = 0.021$) and burn index ($r = 0.70$, $p = 0.008$). The daily urinary excretion of 8-OHdG correlated with the daily urinary excretion of norepinephrine and nitrite plus nitrate (NOx) during the studied period except day 2 post burn, and correlated with the daily urinary excretion of 17-hydroxycorticosteroid (17-OHCS) in days 2, 3, and 7 post burn. These data suggest that oxidative cellular damage correlates with burn severity and neuroendocrine system response modulates inflammation and oxidative cellular damage. Modulation of neuroendocrine system response and inflammation in the treatment in the early phase of burn may be useful to reduce the oxidative cellular damage and to prevent multiple organ failures in patients with extensive burn.

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ing with inflammatory mediators play important roles in many pathophysiological processes, causing cell and tissue damage and organ failures (Cuzzocrea et al. 2001). Over the last several decades, antioxidant therapies have been paid many attentions in burn patients. On the other hand, neuroendocrine system is recognized to participate in defense in a stress situation (Eskandari and Sternberg 2002). It is proved that sympathetic nervous system regulates immune system and associates with inflammatory diseases (Sandars and Kohm 2002). Thermal injury is extremely stressful, but the data characterizing the relationship between immuno-neuro-endocrine response and oxidative stress in thermal trauma patients are unknown. We attempted to investigate the relationships among neuroendocrine system response, inflammation and oxidative cellular damage. Various markers of oxidative damage have been identified. In the past, popular markers, such as malondialdehyde (MDA), oxidized low density lipoprotein (LDL) cholesterol, MDA-modified LDL, anti-antibodies against oxidized LDL, F₂-isoprostanate, and conjugated diene were designed for lipid peroxidation (Halliwell and Kendall 1999; (Morimoto et al. 2005; Tsuboi et al. 2006), but markers for oxidative damage to the cell were few, particularly for the cellular damage in thermal trauma patients. It has been reported that 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a good marker of oxidative cellular damage. 8-OHdG is an abundant base modification in mammalian DNA, increases with oxidative damage, and can be easily detected in urine (Erhola et al. 1997; Honda et al. 2000). Concerning 8-OHdG, the prior studies have focused only on aging, mutation and DNA repair, and cancer (Shigenaga et al. 1989), but very little is known in thermal trauma.

In the present study, we investigated the oxidative DNA damage in burn patients in relation to the changes of neuroendocrine system response and inflammation, and we propose a new approach of treatment for the patients with extensive burn.

**Patients and Methods**

Fourteen burn patients whom we treated in the intensive care units consecutively from December 1999 to April 2004 and whose urine samples were collected from day 1 post burn were enrolled in this study. Fourteen burn patients were eight males and six females aged between 4 and 87 years old (mean ± S.D.: 42.3 ± 31.4 years old). Burns covered between 10-96 percent of total body surface area (%TBSA)(mean ± s.d.: 32.3 ± 27.6 %TBSA), burn index (BI = %TBSA of third degree burn plus half of %TBSA of second degree burn) was 24.4 ± 25.2 (mean ± s.d.), and prognostic burn index (PBI = BI + age) was 66.9 ± 31.3 (mean ± S.D.). Six of them accompanied with inhalation injury, and three patients died on the 20th, 38th, and 64th day post burn (Table 1,  

**Table 1. Characteristics of the burn patients.**

<table>
<thead>
<tr>
<th></th>
<th>14 patients</th>
<th>8 patientsa</th>
</tr>
</thead>
<tbody>
<tr>
<td>male/female</td>
<td>8/6</td>
<td>5/3</td>
</tr>
<tr>
<td>age</td>
<td>42.3 ± 31.4*</td>
<td>48.0 ± 26.0*</td>
</tr>
<tr>
<td>%TBSA</td>
<td>32.3 ± 27.6*</td>
<td>33.4 ± 34.0*</td>
</tr>
<tr>
<td>BI</td>
<td>24.4 ± 25.2*</td>
<td>30.0 ± 33.3*</td>
</tr>
<tr>
<td>PBI</td>
<td>66.9 ± 31.3*</td>
<td>77.6 ± 26.9*</td>
</tr>
<tr>
<td>inhalation injury +/−</td>
<td>6/8</td>
<td>4/4</td>
</tr>
<tr>
<td>died/survival</td>
<td>3/11</td>
<td>3/5</td>
</tr>
</tbody>
</table>

*mean ± S.D.

a The eight patients were treated in ICU without operation and urine samples of these patients were collected in succession in the studied period.

Burn index (BI) = 3rd degree burn (%TBSA) + 1/2 2nd degree burn (%TBSA)
PBI = BI + AGE
Neuroendocrine System and Oxidative Cellular Damage in Burn

Standard treatments were performed throughout the studied period of seven days post burn. Fluid resuscitation was started as soon as possible, sufficient energy support was done after the resuscitation phase, and escharotomy was performed immediately after admission if required. For protecting burn wound from sepsis, tangential or epifascial excision and skin grafting were performed as early as possible. The first operations were performed on day 3 post burn in three patients, on day 5 in one patient, and on day 7 in one patient.

We collected 24-hr urine samples of patients from day 1 post burn to day 7. All urine samples were stored at −80°C until analyzed. The concentrations of epinephrine, norepinephrine, 17-hydroxycorticosteroid (17-OHCS), nitrate plus nitrite (NOx), and 8-OHdG were measured. One patient was discharged from the ICU on day 3, and one patient was transferred to the dermatology ward after the operation on day 3. Likewise, other four patients were undergone operation. So, for the study of the changes of daily urinary excretion of indicators reflecting only burn stress, we adopted eight patients (Table 1, right column), who were treated in the ICU without operation and whose urine samples were collected in succession in the studied period. The correlations between the daily indicators were studied in all samples.

Urinary concentrations of epinephrine and norepinephrine were examined by HPLC (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Sendai), urinary concentrations of 17-OHCS and NOx were measured by Colorimetric Method (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc.). A competitive ELISA kit (Japan Institute for the Control of Aging, Shizuoka) was used for the detection of 8-OHdG. The daily total amounts of the indicators mentioned above were calculated by urine concentration multiplied by the urine volume of 24 hrs. For excluding the influence of body sizes of the patients, the daily total amounts of urinary indicators divided by body weight (kg) were used for analysis.

Statistical analysis

Statistical analysis was carried out using SPSS11.0. All data expressed as mean ± s.d. if not mentioned specially. Correlations between indicators were assessed by Pearson’s correlation. Difference was considered significant at $p < 0.05$.

RESULTS

The relationships between burn severities and the total amounts of urinary excreted indicators of neuroendocrine system response, inflammation, and cellular damage in the first 24 hrs are shown in Table 2. There were positive correlations between %TBSA and urinary excretion of epinephrine ($r = 0.65$, $p = 0.015$), norepinephrine ($r = 0.73$, $p = 0.005$), 17-OHCS ($r = 0.65$, $p = 0.016$), and 8-OHdG ($r = 0.63$, $p = 0.021$). BI also correlated with urinary excretion of epinephrine ($r = 0.74$, $p = 0.004$), norepinephrine ($r = 0.78$, $p = 0.002$), 17-OHCS ($r = 0.73$, $p = 0.005$), and 8-OHdG ($r = 0.70$, $p = 0.008$). However, PBI had no significant correlation with urinary excretion of all indicators.

The changes in daily total amounts of urinary excretion of epinephrine, norepinephrine, 17-OHCS, NOx, and 8-OHdG in eight patients (Table 1, right column), who were treated in ICU without operation and whose urine samples were collected in succession in the studied period, are shown in Figs.1-5. Epinephrine was excreted

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Table 2. Correlations between burn severities and the indicators of neuroendocrine system response and inflammation, inflammation, and oxidative cellular damage excreted in urine in the first 24 hrs post burn.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>%TBSA</th>
<th>BI</th>
<th>PBI</th>
</tr>
</thead>
<tbody>
<tr>
<td>epinephrine</td>
<td>$r = 0.65$, $p = 0.015$</td>
<td>$r = 0.74$, $p = 0.004$</td>
<td>n.s.</td>
</tr>
<tr>
<td>norepinephrine</td>
<td>$r = 0.73$, $p = 0.005$</td>
<td>$r = 0.78$, $p = 0.002$</td>
<td>n.s.</td>
</tr>
<tr>
<td>17-OHCS</td>
<td>$r = 0.65$, $p = 0.016$</td>
<td>$r = 0.73$, $p = 0.005$</td>
<td>n.s.</td>
</tr>
<tr>
<td>NOx</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>8-OHdG</td>
<td>$r = 0.63$, $p = 0.021$</td>
<td>$r = 0.70$, $p = 0.008$</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.s., not statistically significant.
Epinephrine was excreted much on the first day and then decreased gradually. The maximum excretions of norepinephrine, 17-OHCS and NOx were observed on day 3 post burn. Daily urinary excretion of 8-OHdG was not markedly changed during the seven days of investigation, although the values were all above the standard value.

On the first day, the urinary excretion of 8-OHdG correlated strongly with excretion of norepinephrine ($r = 0.83$, $p < 0.001$) and NOx ($r = 0.90$, $p < 0.001$) (Table 3). Urinary excretion of norepinephrine also had correlation with excretions of 17-OHCS ($r = 0.70$, $p = 0.005$) and NOx ($r = 0.74$, $p = 0.002$). During the studied period, the excretion of 8-OHdG remained to correlate with the excretion of norepinephrine and NOx from day 3 to day 7 post burn, and correlated with the excretion of 17-OHCS in days 2, 3, and 7 post burn (Table 3). There was no significant correlation between the urinary excretion of 8-OHdG.
The maximum excretion of NOx was observed on day 3 post burn. (Standard value of daily total amount of urinary excretion of NOx is 284-369 μMol/day (Marzinzig et al. 1997), that is 6.5-8.5 μMol/kg/day for a man weighted 65 kg and urinates 1,500 ml/day.)

**TABLE 3.** Correlations among the urinary excretion of 8-OHdG and the urinary excretions of indicators of neuroendocrine system response and inflammation.

<table>
<thead>
<tr>
<th>8-OHdG</th>
<th>day 1 (n = 14)</th>
<th>day 2 (n = 14)</th>
<th>day 3 (n = 11)</th>
<th>day 4 (n = 12)</th>
<th>day 5 (n = 11)</th>
<th>day 6 (n = 12)</th>
<th>day 7 (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>epinephrine</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>norepinephrine</td>
<td><em>r</em> = 0.83</td>
<td><em>r</em> = 0.75</td>
<td><em>r</em> = 0.80</td>
<td><em>r</em> = 0.76</td>
<td><em>r</em> = 0.85</td>
<td><em>r</em> = 0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>p</em> &lt; 0.001</td>
<td><em>p</em> = 0.008</td>
<td><em>p</em> = 0.002</td>
<td><em>p</em> = 0.024</td>
<td><em>p</em> = 0.003</td>
<td><em>p</em> &lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>17-OHCS</td>
<td>n.s.</td>
<td><em>r</em> = 0.55</td>
<td><em>r</em> = 0.66</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td><em>r</em> = 0.62</td>
</tr>
<tr>
<td></td>
<td><em>p</em> = 0.044</td>
<td><em>p</em> = 0.027</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td><em>p</em> = 0.039</td>
<td></td>
</tr>
<tr>
<td>NOx</td>
<td><em>r</em> = 0.90</td>
<td>n.s.</td>
<td><em>r</em> = 0.79</td>
<td><em>r</em> = 0.78</td>
<td><em>r</em> = 0.70</td>
<td><em>r</em> = 0.91</td>
<td><em>r</em> = 0.80</td>
</tr>
<tr>
<td></td>
<td><em>p</em> &lt; 0.001</td>
<td><em>p</em> = 0.004</td>
<td><em>p</em> = 0.003</td>
<td><em>p</em> = 0.034</td>
<td><em>p</em> &lt; 0.001</td>
<td><em>p</em> = 0.002</td>
<td></td>
</tr>
</tbody>
</table>

Two patients were discharged from the ICU on day 3 post burn. The urine samples of three patients out of the remained twelve patients were accidentally missed on day 3, 5, and 7. n.s., not statistically significant.

and the urinary excretion of epinephrine in the studied period.

Figs. 6, 7, and 8 show the positive correlations between the 8-OHdG excretion and the excretions of norepinephrine, 17-OHCS, and NOx during seven days, respectively.
DISCUSSION

Major thermal injury produces pathophysiological changes in the external and the internal systems of the body, and the initial life-threatening alteration can occur in the internal environment. It has been recognized that the changes of neuroendocrine system are the earliest response to stress. Within seconds after the stress, enhanced secretion of catecholamines (epinephrine, norepinephrine, and dopamine) from the sympathetic nervous system occurs, and corticotrophin-releasing hormone (CRH) is released into the portal circulation from hypothalamus and enhances secretion of pituitary adrenocorticotropic hormone (ACTH). In the case of the hemorrhage, the first wave of neuroendocrine response also includes massive secretion of arginin vasopression (AVP) from the pituitary and renin from the kidney (Hench et al. 1950; Munck et al. 1984; Marzinzig et al. 1997; Sabatini et al. 2005). From hours to days after the stress, glucocorticoid hormone and sympatho-adreno-medullary system play important roles in corresponding with each other (Ingle 1952; Orchinik et al. 1991; Munck and Naray-Fejes-Toth 1992). Our data supported these findings, and excretion of catecholamines and 17-OHCS in the first day had significant positive correlation with the severities of burn. Recently, it has been reported that proinflammatory cytokine such as TNF-α induces activation of hypothalamic-pituitary-adrenal axis (Buller 2001) and inflammatory cells have receptors such as α-7
nicotinic acetylcholine receptor which match for neurological stimuli (Wang et al. 2003). In our study, in day 1 post burn, the severity of burn did not correlate with the urinary excretion of NOx, the indicator of inflammation, which increased later and most on day 3 post burn.

It is known that major trauma produces abundant free radicals and impairs free radical scavenging mechanisms. We first found that there were significant relationships between burn severities and total amount urinary excretion of 8-OHdG, and 8-OHdG had significant correlations with neuroendocrine system response and inflammation in burn patients. 8-OHdG is an oxidized nucleoside of DNA, which is an important biomarker for the evaluation of oxidative DNA damage. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) produce 8-hydroxylation of guanine base, and the damaged DNA is repaired in vivo by endonucleases. Nuclear and mitochondrial DNA from tissue and blood lymphocyte is usually the site of oxidation damage (Yamamoto et al. 1992). Among all purine and pyridine bases, guanine is most prone to oxidation. Upon oxidation a hydroxyl group is added to the 8th position of the guanine molecule and the oxidatively modified product 8-OHdG is one of the predominant forms of free radical-induced lesions of DNA. Oxidative modified DNA in the form of 8-OHdG can be quantified to indicate the extent of DNA damage (Szabo and Ohshima 1997). 8-OHdG dissolvable in free water is excreted into the urine without further metabolism (Kasai et al. 1986; Moore and Orchinik 1994; Munck and Naray-Fejes-Toth 1994). Numerous studies have reported that 8-OHdG links pathogenically to a variety of chronic aging-associated degenerative diseases such as cancer, coronary heart disease, and diabetes, but an oxidative DNA damage caused by an acute trauma such as thermal injury remains unclear. The present study suggests that oxidative cellular damage evaluated by the urinary excretion of 8-OHdG correlates with the severities of burn and the oxidative cellular damage has something to do with neuroendocrine system response.

We also found that there were strong correlations between the 8-OHdG excretion and the excretion of NOx, one of inflammatory mediators, throughout the early phase of burn. This indicates that nitric oxide (NO) plays a main role in production of 8-OHdG in the early phase of burn. Little is known about the mechanisms of induction of 8-OHdG in the thermal stress. It has been clear that a significant inflammatory response including up-regulation of the inducible nitric oxide synthase (iNOS) occurs during the first hour after the injury, and iNOS-mediated NO production is profoundly up-regulated (Chung et al. 1991). NO has important effects on cellular respiration and mitochondrial ROS production, which could either contribute or exacerbate cellular damage (Rhee et al. 1998). In the presence of oxidative stress, RNS and ROS generated in vivo can cause oxidative damage to lipids, proteins, and nucleic acids. NO can injury cells directly or by reaction with superoxide (O\textsuperscript{2-}) to form the oxidant peroxynitrate (ONOO\textsuperscript{-}) (Rhee et al. 1998).

Burn trauma produces significant fluid shifts that reduce cardiac output and tissue perfusion and thus causes ischemia of the tissues (Cakir and Yegen 2004). While aggressive volume replacement increases tissue ischemia in the early phase, and in turn, oxygen delivery by reperfusion in previously ischemic tissue occurs in the latter phase. This restoration of oxygen delivery is thought to initiate a series of deleterious events that exacerbate tissue injury though oxygen free radical formation via xanthine oxidase as well as oxidative stress resulted from inflammatory cells activated by proinflammatory cytokines (Giroir et al. 1994; Horton et al. 1998; Cain et al. 1999; Horton 2003). Till et al. (1989) reported that excision of the burned skin immediately after thermal injury, as early as 15 min after thermal trauma, significantly diminished the increase in plasma xanthine oxidase activity.

Thermal injury leads to the activation of multiple host mediator systems. Enhancing releases of catecholamines and glucocorticoid play crucial roles in the adaptation of the organ to stress in the early phase of burn. The secretion of glucocorticoid is a classic endocrine response to stress. 17-OHCS represents the last metabolism
materials of glucocorticoid excreted in urine. The relative delay in elevating glucocorticoid reins the stress-activated defense reactions, prevents them from overshooting, and maintains the homeostasis of internal environment (Cooke et al. 2002; Li and Jackson 2002). The effects of glucocorticoid on permission of stressor and suppression of defense response may play a crucial role in survival and cellular damage. Meanwhile, NO has also dual role as a protective or toxic molecule depending on several factors, such as the isoform of NOS involved, the amount of NO, and the type of cells in which NO is synthesized. In early phase of burn, a small amount of NO produced via constitutive NOS (cNOS) exerts protective effect on cell function. In addition, a small amount of NO serves an important vasodilatory role in the peripheral circulation and prevents platelet and neutrophil to adhere to the microvasculature (Cooke et al. 2002). It was reported that fluid resuscitation containing arginine, a precursor of NO, in burn trauma provided myocardial protection, prevented platelet and neutrophil adhesion, and provided organ protection (Horton et al. 1998).

In summary, we suggest that DNA damage strongly correlates with burn severity, neuroendocrine system response, and inflammation in the early phase of burn. And as far as this study is concerned, NO does not seem to act favorably in burn trauma. These results lead us to propose that the suppression of neurological stress and inflammatory response and the enhancement of endocrine system would be recommended to protect the vital organs from oxidative challenges in severe burn patients. Administration of glucocorticoid and/or iNOS inhibitors can be useful in prevention of oxidative stress.

References


nitrates, and S-nitrosothiols. *Nitric Oxide*, 1, 177-189.


