Soluble E-cadherin concentrations in patients with systemic inflammatory response syndrome and multiorgan dysfunction syndrome

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Summary
We have assessed the role of the cell–cell adhesion molecule, E-cadherin, in the pathogenesis of multiorgan failure in 24 intensive care patients with sepsis and varying degrees of organ dysfunction, compared with 21 healthy subjects. Plasma soluble E-cadherin (sE-cadherin) was measured by enzyme immunoassay. The median concentration of sE-cadherin in normal subjects was 3.21 μg ml⁻¹ compared with 6.00 μg ml⁻¹ in patients with sepsis and organ dysfunction (P = 0.0019). There was no statistically significant difference in concentrations of sE-cadherin in survivors compared with nonsurvivors. Concentrations of sE-cadherin tended to increase with the severity of organ failure. We conclude that sE-cadherin is increased in inflammation and injury, and may be related to the degree of multiorgan failure after sepsis. (Br. J. Anaesth. 1996; 76: 629–631)

Key words

Sepsis, septic shock and multiorgan failure are important contributors to mortality and morbidity in patients in the intensive care unit [1–3]. Shock and infection are the two most common predisposing factors in the development of multiorgan dysfunction [3], causing an inflammatory response initiating a cascade of events leading to widespread endothelial damage [2, 4, 5] and failure of the microcirculation. Adhesion molecules are important in cell–cell and cell–matrix interactions. Integrins and selectins play fundamental roles in the interaction between leucocytes and endothelial cells [6, 7]. Cadherins mediate cell–cell adhesion and are important in maintaining tissue architecture and consequently organ function. E-cadherin is a cell adhesive molecule found in a variety of tissues [8]. Soluble forms of integrins, selectins and cadherins have been identified.

Increased concentrations of immunoreactive soluble forms of endothelial adhesion molecules have been found in patients with sepsis and multiorgan dysfunction [9–11]. Increased concentrations of soluble E-cadherin (sE-cadherin) have been reported in patients with various cancers [8, 12]. There has been no previous study on the role of E-cadherin in patients with sepsis. Circulating sE-cadherin may reflect tissue turnover or regeneration, and may be important in the regulation of normal organ function. We have measured plasma sE-cadherin concentrations in patients with sepsis and varying degrees of multiorgan dysfunction.

Patients and methods
After approval by the local Clinical Research Ethics Committee, we studied 24 consecutive patients admitted to the intensive care unit, who were more than 18 yr of age, when they fulfilled the Bone sepsis criteria [13]. Fourteen patients were medical patients, nine were postoperative and one was post-trauma. Blood samples were obtained from an indwelling arterial cannula and collected into a heparinized tube. APACHE II score and numbers of dysfunctioning organs (table 1) [14] were recorded at sampling. In four patients sequential samples were obtained when patients developed increasing numbers of dysfunctional organs. After immediate centrifugation, plasma was separated into aliquots and stored at −20°C for assay of sE-cadherin. Venous blood samples were also obtained from 21 healthy subjects (11 male) aged 21–54 yr.

Samples were assayed for sE-cadherin using a commercially available sandwich enzyme immunoassay (Takara Shuzo Co. Ltd, Japan). Briefly, a microtitre plate was coated with a mouse monoclonal anti-human E-cadherin antibody, non-specific binding was blocked using a blocking buffer and diluted samples and standards were added to the wells. After incubation and washing steps, an anti-mouse IgG antibody conjugated with horseradish peroxidase was then added to each well, incubated, washed and bound sE-cadherin detected by incubation with substrate (o-phenylenediamine). After the addition of stop solution, the absorbances of the wells were measured at 490 nm using a Titertek Multiskan MCC reader and the concentration of sE-cadherin determined by comparison with the standard curve.
using Flexicalc software (Wallac, Finland). All standards and samples were assayed in duplicate. The coefficient of variation (CV) of replicate samples was less than 10% and a between-plate CV based on a sample with a mean value of 3.3 μg ml⁻¹ was 13.1% (n = 4).

Data were analysed using Microsoft Excel 5.0 with Astute statistical software add-in. When tested for normality using the Komolgorov–Smirnov goodness-of-fit test and normal plots, the data were found to be non-normally distributed and accordingly non-parametric statistical tests were used. Comparison of sE-cadherin concentrations at study entry between survivors and non-survivors, and healthy subjects was made using the Mann–Whitney U test. Pearson rank linear regression was used to assess the relationship between APACHE II score and sE-cadherin concentrations. Kruskal–Wallis ANOVA was used to assess the relationship between sE-cadherin concentrations and the degree of organ failure. P < 0.05 was considered statistically significant.

Results

We studied 24 patients, median age 65 (range 19–79) yr, 18 of whom were male. The median APACHE II score at the time of sampling was 26 (13–39). Sixteen patients died (67%). The median APACHE score in those who died was 25.5 (16–37) and 20.5 (13–31) in survivors (ns). Immunoreactive sE-cadherin concentrations were significantly higher in patients with sepsis and organ dysfunction (6.00 (0.98–19.98) μg ml⁻¹) than those in healthy subjects (3.28 (1.18–6.88) μg ml⁻¹) (P = 0.0019) (fig. 1). There was a tendency for sE-cadherin concentrations to increase with the degree of organ dysfunction, although this failed to reach statistical significance (fig. 2). There was no difference in sE-cadherin concentrations between patients who died (5.53 (2.10–19.98) μg ml⁻¹) and those who survived (8.63 (0.98–11.98) μg ml⁻¹) (P = 0.86). sE-cadherin concentration did not correlate with APACHE II score (r = 0.25, P = 0.25).

Discussion

We have demonstrated significantly increased concentrations of the cell–cell and cell–matrix adhesion molecule, soluble E-cadherin, in patients with sepsis and organ dysfunction. Plasma concentrations tended to correlate with increasing severity of illness, as reflected by the increasing numbers of organ systems failing. It is possible that these increased concentrations reflect the severity of tissue damage and degree of epithelial turnover and may suggest a role for abnormalities in intercellular adhesion in the pathogenesis of multiorgan dysfunction and failure.

Cadherins play an essential role in normal growth and tissue development. There have been three forms described, E-, N- and P-, with characteristic tissue distribution [8]. They share a common primary structure and all mediate cell–cell adhesion and maintenance of normal tissue architecture. Several studies have shown that loss of cell surface immunoreactive E-cadherin is associated with tumour progression and tissue invasion [8, 12]. Soluble forms of E-cadherin have been identified in plasma representing functionally active degradation fragments and may indicate either regeneration or loss of cell surface E-cadherin.

We suggest that increased sE-cadherin concentrations represent increased cleavage of tissue E-cadherin secondary to increased protease activity [15] or free radical release [14] from activated
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neutrophils resulting in loss of tissue architecture and function. However, it is possible that there is a generalized increase in epithelial cell turnover with an associated requirement for E-cadherin regeneration. \textit{In vitro}, interleukin-1 causes shedding of biologically active E-selectin (an endothelial cell adhesion molecule) from the cell surface of cultured human endothelial cells [10]. It is not clear if cytokines present in the circulation of septic patients as a result of the inflammatory process can cause similar shedding of other adhesion molecules.

In the present study, measured immunoreactive sE-cadherin concentrations may have been underestimated as high concentrations of proteolytic enzymes may have yielded smaller fragments which may not have been detected in the assay system used. However, concentrations in our patients with sepsis were generally higher than those reported previously with various tumours [12]. Moreover, marked differences were found between plasma from patients with sepsis and that from healthy subjects.

In summary, we have shown increased circulating concentrations of sE-cadherin in patients with sepsis and varying degrees of multiple organ failure. Further studies are required to elucidate the mechanism of shedding and the pathophysiological role of E-cadherin in such patients.

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References