Elevated Plasma Endothelin-1 Concentrations Are Associated with the Severity of Illness in Patients with Sepsis

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Plasma immunoreactive endothelin-1 concentrations were determined by radioimmunoassay in 11 septic patients during the first 24 hours after the development of the sepsis syndrome in 15 nonseptic postoperative patients studied 24 hours after open heart surgery and in 14 healthy volunteers. Mean endothelin-1 plasma concentrations were significantly ($p < 0.001$) increased in septic patients (19.9 ± 2.2 pg/mL, mean ± standard error) compared to concentrations found in postoperative cardiac patients (11.9 ± 0.7 pg/mL) or in healthy volunteers (6.1 ± 0.3 pg/mL). In septic patients elevated plasma concentrations of endothelin-1 were inversely correlated with cardiac index ($r = -0.80$, $p < 0.005$) and positively correlated the severity of illness as documented by APACHE II score ($r = 0.74$, $p < 0.01$) and plasma creatinine levels ($r = 0.80$, $p < 0.005$). No such correlations were found in postoperative cardiac patients. These results indicate that endothelin-1 concentrations are correlated with the severity of illness and depression of cardiac output in patients with sepsis.

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ENDOTHELIN, A NEWLY described potent vasoconstrictor peptide[1] recently was isolated from the culture supernatant of porcine[2] and human[3] endothelial cells. In animal studies administration of exogenous endothelin-1 was reported to produce intense vasoconstriction in various vascular beds[4–7] and to induce the release of prostacyclin and endothelium-derived relaxing factor.[8] We recently demonstrated that plasma and pulmonary lymph concentrations of endothelin-1–like immunoreactivity (LI) were increased during sustained endotoxemia in sheep, whereas no detectable plasma or lymph levels of endothelin-1–LI were found in normal sheep.[9] Endotoxin-induced endothelial cell injury is the most probable cause of the observed increased circulating endothelin-1–LI concentration. The endogenous release of this vasoconstrictor peptide and its possible role in sepsis have not been studied yet in humans, whereas some evidence was produced that endothelin may be involved in the pathophysiology of acute myocardial infarction[10] and in uremia[11] in humans. In the present study, we measured the plasma endothelin-1–LI in patients with a sepsis syndrome[12] and compared it with plasma endothelin-1 concentrations measured in patients after open heart surgery and in healthy human volunteers.

Methods

Eleven patients with a diagnosis of the sepsis syndrome[12] (age, 56 ± 6 years [mean ± standard error]; range, 21 to 75 years; 76 ± 5 kg body weight, Table 1), 15 nonseptic patients (age, 53 ± 8 years; range, 24 to 83 years; 75.4 ± 1.7 kg body weight) studied 24 hours after open heart surgery, and 14 healthy volunteers (age, 44 ± 6 years; range, 32 to 55 years; 72.5 ± 2.1 kg body weight) were included in the study. The study was approved by the Committee for Ethics in Human Research of our institution and informed consent was obtained from all patients and volunteers.

In patients with the sepsis syndrome, arterial blood samples were taken during the first 24 hours after the diagnosis of the septic syndrome. The APACHE II score,[13], derived from 12 clinical and laboratory variables, age, and previous health status were determined for all

Supported in part by the Swiss National Funds for Scientific Research grants 3-749.0.87 and 32.25205.88, the Swedish Medical Research Council (grant 144-6554), and the American Council for Tobacco Research.

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Accepted for publication May 23, 1990.

TABLE 1. *Characteristics of Patients*

Patient	Age (yrs)	Sex (M/F)	Weight (kg)	APACHE II score	Blood cultures	Creatinine μmol/L	WBC $\times 10^9$/L	PLT $\times 10^9$/L	Diagnosis	Outcome
1	38	M	78	15	—	34	25.0	107	Acute pancreatitis, peritonitis	D
2	74	M	90	29	*E. coli*	424	18.0	15	Infected hip prosthesis	S
3	54	M	74	14	—	82	14.6	36	Postop Ca esophagus, ARDS	D
4	65	M	78	14	*Staph. aureus*	55	16.9	104	Postop Ca pancreas, peritonitis	D
5	75	M	50	20	—	164	15.0	243	Acute peritonitis	D
6	67	M	84	27	Proteus	266	3.5	5	Postop meningitis	D
7	38	M	75	7	*Staph. aureus*	86	6.0	69	Multiple trauma, ARDS	S
8	21	F	60	15	*Staph. aureus*	55	3.0	54	Multiple trauma, ARDS	S
9	76	M	86	26	Pseudomonas	398	20.1	210	Postop Ca bile duct, peritonitis	S
10	63	M	95	21	*Staph. aureus*	357	13.9	47	Ruptured aortic aneurysm	S
11	60	M	80	15	*Staph. aureus*	247	26.4	82	Acute pancreatitis	S

WBC, white blood cell count; PLT, platelets; ARDS, adult respiratory distress syndrome; Ca, carcinoma; D, died; S, survived.

septic patients at the time of admission to the intensive care unit. None of the patients were receiving vasoactive or inotropic drugs, except for a low dose of dopamine (2 to 3 μg/kg • min). Patients studied after open heart surgery were not receiving any vasoactive or inotropic support at the time of the study and had been hemodynamically stable for at least 8 hours before blood samples were taken. Blood samples were collected in tubes containing 5 mg of edetic acid (ethylenedinitrilo tetraacetic acid) and the plasma separated by centrifugation. Plasma samples were stored at −70 C until analysis.

After extraction of plasma in acid-ethanol, endothelin-1–LI in plasma was determined by radioimmunoassay using a specific antiserum (diluted 1:60,000) raised in rabbits against porcine endothelin-1. The cross-reactivity to other endothelin peptides are (endothelin-1 expressed as 100%): endothelin-2, 64%; endothelin-3, 7%; and big endothelin-1, 90%. The antiserum shows no cross-reactivity to other peptides such as atrial natriuretic peptide, vasopressin, or angiotensin I, II, and III. Endothelin-1 labeled with ^{125}I was used as tracer and porcine endothelin-1 as a standard. The assay was incubated at +4 C in 0.1 mol/L (molar) phosphate buffer, pH 7.4, containing 0.1% bovine serum albumin and 0.1% Triton-X-100. Bound and free fractions were separated using a secondary antibody.[14] The detection limit of the assay is 0.75 fmol/tube.

All recorded variables (mean ± SE) were compared between the three groups of patients and volunteers using a one-way analysis of variance followed by Duncan's multicomparisons test. A p value < 0.05 was considered statistically significant.

Results

Hemodynamic, respiratory, and plasma lactate values of patients are summarized in Table 2. Plasma endothelin-1–LI concentrations for both groups of patients and healthy volunteers are shown in Figure 1. In volunteers, the mean plasma endothelin- 1–LI level was 6.1 ± 0.3 pg/mL. Patients studied 24 hours after open heart surgery had a significantly increased mean plasma concentration of endothelin-1–LI compared to volunteers (11.9 ± 0.7 pg/mL, $p < 0.0001$). The mean plasma endothelin-1–LI level measured in patients with the sepsis syndrome also was significantly increased (19.9 ± 2.2 pg/mL) compared to volunteers ($p < 0.0001$) as well as compared to postoperative cardiac patients ($p = 0.0004$). No difference in plasma endothelin-1–LI level was observed in the septic group between patients with or without positive blood cultures. However the three patients with a gram-negative septicemia had a significantly greater plasma level of endothelin-1–LI than the other patients in the septic group (26.8 ± 4.6 *versus* 17.3 ± 1.8 pg/mL, $p < 0.005$). The relationship between plasma endothelin-1–LI concentrations and cardiac index for both patient groups is shown in Figure 2. In septic patients, there was a significant inverse linear correlation between plasma endothelin-1–LI levels and cardiac index ($r = -0.80$, $p < 0.005$). In these patients endothelin-1–LI plasma concentrations were significantly correlated with APACHE II score ($r = 0.74$, $p < 0.01$) and plasma creatinine levels ($r = 0.80$, $p < 0.005$), but not with plasma lactate levels, mean systemic arterial pressure, systemic vascular resistance index, intrapulmonary venous admixture, or alveolar–arterial oxygen gradient/inspired oxygen fraction ratio. In postoperative cardiac patients, no such correlations were found between plasma endothelin-1–LI levels and cardiac index (Fig. 2), plasma creatinine levels, and other hemodynamic or respiratory variables.

Discussion

The results of the present study show that plasma endothelin-1–LI levels are significantly increased in patients with the sepsis syndrome compared to the values found in postoperative nonseptic patients or in healthy volunteers. These data also provide evidence that in septic pa-

TABLE 2. *Hemodynamic, Respiratory, Plasma Lactate, and Endothelin Values of Patients*

Patient	MAP (mmHg)	MPAP (mmHg)	CI ($l \cdot min^{-1} \cdot m^{-2}$)	CVP (cm H_2O)	PCWP (cm H_2O)	$AaDO_2/FIO_2$ (kPa)	Qs/Qt (%)	Lactate (mmol/L)	Endothelin (pg/mL)
Patients with a septic syndrome									
1	77	36	7.02	14	16	67	60	1.06	14.27
2	56	24	2.57	12	10	69	30	2.70	23.29
3	78	29	5.91	11	12	57	30	2.60	16.57
4	61	17	6.18	8	8	69	44	3.52	15.32
5	67	25	4.00	9	14	48	30	2.37	18.71
6	78	25	3.57	6	11	57	29	6.36	21.20
7	81	27	7.08	13	11	59	33	1.35	7.85
8	72	31	5.22	9	8	68	40	3.56	17.79
9	63	23	3.35	14	20	51	25	2.84	35.87
10	59	27	4.07	12	16	60	29	1.95	23.02
11	55	24	3.06	12	11	67	29	6.15	24.56
x ± SE	68 ± 3	26 ± 1	4.7 ± 0.5	11 ± 1	12 ± 1	61 ± 2	35 ± 3	2.8 ± 0.5	19.9 ± 2.2
Nonseptic postoperative cardiac patients									
x ± SE	77 ± 2*	22 ± 2	2.4 ± 0.1*	12 ± 1	13 ± 1	35 ± 2*	19 ± 1*	1.7 ± 0.2*	11.9 ± 0.7*

* $p < 0.05$ from septic patients.

n = 11 in septic patient group and n = 15 in nonseptic postoperative cardiac patients.

MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; CI, cardiac index; CVP, central venous pressure; PCWP, pulmonary capillary wedge pressure; $AaDO_2/FIO_2$, alveolar arterial oxygen gradient/inspired oxygen fraction; Qs/Qt, intrapulmonary venous admixture.

tients the plasma concentration of endothelin-1–LI correlates with the severity of illness. We found that patients with the greatest severity of disease at the time of the study as measured by the APACHE II score had the highest levels of endothelin-1–LI. In addition, we found an inverse correlation between plasma endothelin-1–LI levels and cardiac index for comparable cardiac filling pressures (Fig. 2). It has been proposed that an elevated cardiac output is needed in septicemia to maintain an effective oxygen delivery.[15] In the present study, patients with the greatest endothelin-1–LI levels had the highest APACHE II scores, but their cardiac index was not elevated. The fact that there was an inverse correlation between plasma endothelin-1–LI levels and cardiac index, but no significant relationship between plasma endothelin-1–LI levels and systemic vascular resistance index suggests that in humans with sepsis, endothelin-1 might be one of the

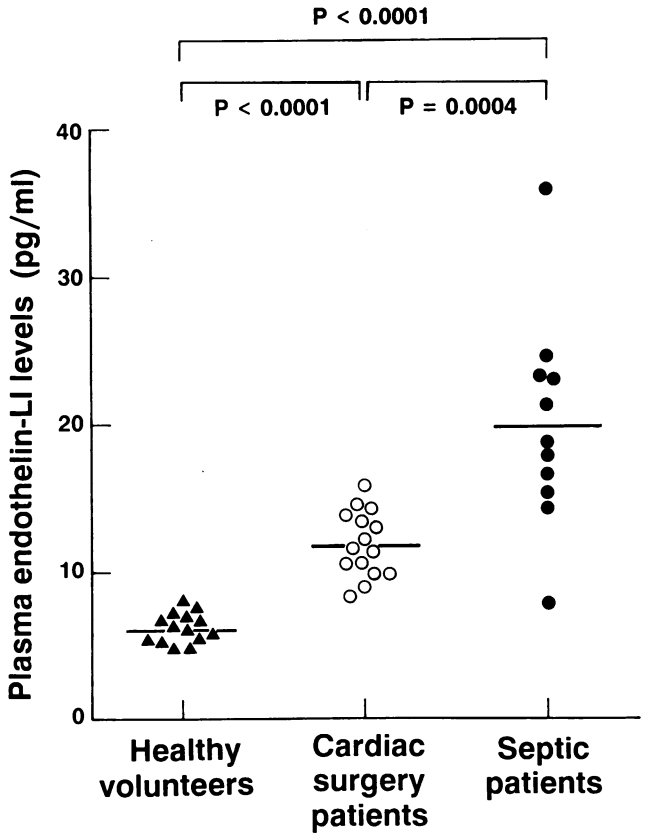

FIG. 1. Plasma endothelin concentrations.

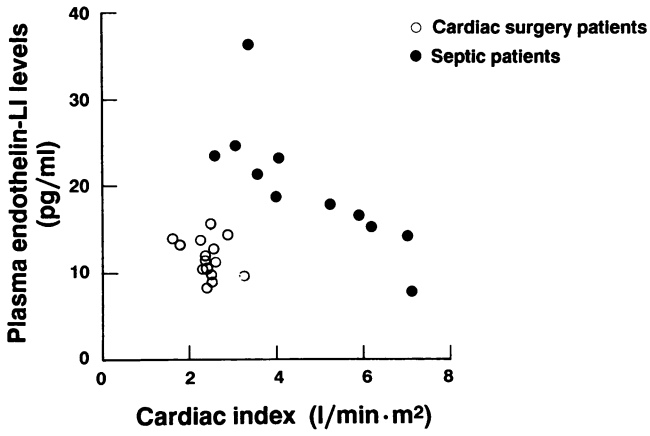

FIG. 2. Relationship between plasma endothelin concentrations and cardiac index in both groups of patients.

described circulating myocardial depressant substances[16] rather than acting as an effective systemic vasoconstrictor. Interestingly sarafotoxins that represent endothelinlike peptides in some snake venom exert strong cardiac toxicity due to prolonged coronary vasospasm.[17] Increased plasma and cardiac tissue concentrations of endothelin-1 were also reported in an experimental model of myocardial infarction.[18] Neutralizing endogenous endothelin-1 with administration of monoclonal antibodies against endothelin-1 resulted in a significant decrease of the infarction size, indicating that endogenous endothelin-1 is one of the factors that contribute to the extension of myocardial infarction.[18] In our study high levels of endothelin-1–LI were associated with increased plasma creatinine levels and it has been suggested that endothelin-1 also may play an important role in the development of acute renal failure.[19,20]

Increased plasma endothelin-1–LI concentrations measured in septic patients probably are not only due to an acute stress response, as has been reported after major abdominal surgery.[21] Endothelin-1–LI levels in these patients were significantly greater than those measured in patients after major cardiac surgery with extracorporeal bypass and in healthy volunteers. Plasma endothelin-1 concentrations measured in our volunteers were similar to that recently found by Davenport et al.[22] Elevated plasma endothelin-1–LI in sepsis is probably the result of an endotoxin-induced release of endothelin-1 by injured endothelial cells. Previously it was reported in rats that endotoxin stimulates endothelin release *in vivo*.[23] Similarly we found in awake sheep a clearcut increase of endothelin-1–LI levels in pulmonary lymph and plasma during sustained endotoxemia.[9] It also has been demonstrated that *E. coli* endotoxin induces the production of endothelin from cultured bovine endothelials cells.[23] Gram-negative bacteria may themselves produce precursors of endothelin.[24] These animal and *in vitro* results correlate with our human data in which the three patients with gram-negative septicemia had the highest plasma endothelin-1–LI levels. In conclusion our investigation demonstrates that endothelin-1–LI concentrations are correlated with the severity of illness and depression of cardiac output in patients with sepsis. Further clinical studies are needed to define better the precise role of endothelin in the pathophysiology of sepsis.

Acknowledgment

The authors thank Julian Lopez for his technical assistance with this study.

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