CLINICAL STUDY

Serum concentrations of activin and follistatin are elevated and run in parallel in patients with septicemia

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Abstract

Objective: Activin is a growth and differentiation factor of many cell types, and has recently been implicated in inflammatory processes. Clinical data linking activin and its binding protein, follistatin (FS), are lacking. We measured serum levels of activin and FS in patients diagnosed with septicemia. **Patients and measurements:** Eight male and seven female patients of different ages, various forms of septicemia and different clinical outcome were investigated and compared with age- and sex-matched healthy controls. Serum concentrations of FS, activin, C-reactive protein (CRP) and blood leukocyte counts were determined during septicemia.

Results: The median of the maximum activin concentrations of septicemic patients was 3.9-fold higher than in age- and sex-matched healthy control subjects ($P < 0.01$); the median of the maximum FS concentrations was 2.6-fold higher ($P < 0.01$). The highest increase of activin in septicemic patients was approximately 15.8-fold, whereas FS increased by up to 13.2-fold above normal. FS, activin and CRP serum levels generally paralleled each other, but were not correlated with leukocyte counts or clinical outcome.

Conclusions: Circulatory concentrations of activin and FS are elevated in patients diagnosed with septicemia, consistent with potential roles in the systemic inflammatory response.

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Introduction

Activin affects the growth and differentiation of many cell types, stimulates the secretion of follicle-stimulating hormone from the pituitary gland and inhibits growth hormone, prolactin, and adrenocorticotropin release (1–3). Follistatin (FS) specifically binds to activin. As a result, circulating FS 315 neutralizes activin activity by preventing the interaction of the cytokine with its type II receptors (4) and, furthermore, cell surface-bound FS 288 facilitates the lysosomal degradation of activin (5). Both FS and activin mRNAs show a broad tissue distribution (6–8). FS and activin are detectable in serum (9–18), and their concentrations in serum increase with age (18, 19). At present, however, the sources of FS and activin in serum are unknown. Current data suggest that tissue-specific balances of FS and activin govern the growth and differentiation of responsive cell types in an autocrine/paracrine manner (for reviews see 20, 21).

We and others have documented an emerging role for activin and FS in the body's innate immune

response. For instance, activin and FS are secreted by various cell types in response to inflammatory compounds *in vitro* (22–30). Moreover, in some examples of inflammatory processes such as wound healing, inflammatory bowel disease and rheumatoid arthritis, increased activin and/or FS expression has been noted (31–33). While we have reported previously that serum FS concentrations were elevated in patients with septicemia (34), there are few if any data published on activin profiles in clinically important inflammatory syndromes. Therefore, in this study we examined both activin and FS serum responses in patients who were hospitalized and undergoing treatment for septicemia.

Materials and methods

As part of their routine clinical management, serial blood samples were collected from seven female and eight male patients of different ages who suffered from septic infections of different grades of severity. After completion of the clinical routine analyses, FS

and activin were measured in the remnants of serum samples. Since the patients were critically ill, no extra blood samples were drawn for the purposes of this study. The samples were stored frozen at -20°C until assayed. Since FS and activin serum levels increase with age (18, 19), serum samples from age- and sex-matched healthy volunteers served as controls. All samples from diseased and healthy persons were treated in the same way.

Patients were categorized for septicemia according to the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Consensus criteria (manifestation of two or more of the following clinical conditions: body temperature $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$; heart rate >90 beats/min; respiratory rate >20 breaths/min or $\text{PaCO}_2 <32$ mmHg; white blood cell count $>12\,000$ cells/mm³, <4000 cells/mm³, or $>10\%$ immature forms) (35). For 12 of 15 patients the diagnosis of septicemia was proven by culture of the infectious organism from blood. In three cases the culture of the infectious organism failed due to rapid implementation of antibiotic treatment.

Activin A concentrations in serum were measured using a specific ELISA which detects both FS-bound and free activin (13), with the following modifications. The standard used was human recombinant (hr) activin A as described previously (14). The assay sensitivity was 0.1 ng/ml and the intra- and interassay coefficients of variation were 4.7% and 7.8% respectively. Serum samples were assayed against the standard diluted in 5% bovine serum albumin in phosphate-buffered saline (0.01 mol/l). FS concentrations in serum were measured with a radioimmunoassay validated for human FS as previously described (36).

The standard employed was hrFS 288, but the assay cross-reacts with hrFS 315 (35.9%). The assay sensitivity was 2.0 ng/ml and the intra- and interassay coefficients of variation were both $<4.9\%$. The assay measures total FS (free and bound). Numbers of leukocytes, serum creatinine levels, and serum C-reactive protein (CRP) levels were determined by clinical routine methods in the Department of Clinical Chemistry of the University of Göttingen.

Differences in the serum concentrations of FS and activin between septic patients and matched healthy volunteers were analyzed by paired *t*-test. Correlations between measured parameters were calculated with Pearson correlation. The software was Graph Pad Prism 3.0 (Graph Pad, San Diego, CA, USA).

Results and discussion

Peak activin and FS serum concentrations of patients with septicemia were elevated compared with concentrations in age- and sex-matched controls (Table 1). The median of the maximum activin concentration of septicemic patients was 3.9-fold higher than the median in healthy controls ($P < 0.01$); the median of the maximum FS concentrations of septicemic patients was 2.6-fold higher than the median of the FS concentrations in healthy controls ($P < 0.01$). The magnitude of the activin and FS increase during septicemia varied among individuals and there was no close association between FS/activin serum concentrations and clinical outcome.

Figure 1 depicts the individual profiles of serum activin and FS in the seven female (A) and eight male

Table 1 Comparison of activin and FS serum concentrations from patients with septicemia and sex- and age-matched healthy controls.

Number	Diagnosis	Age	Sex	Septicemia (ng/ml)		Control (ng/ml)		Outcome
				Activin	FS	Activin	FS	
1	Sepsis (Staph. aureus)	64	f	0.42	25.77	0.15	4.84	Deceased
2	Sepsis & meningitis (Strep. pneu.)	72	f	0.65	14.48	0.18	10.4	Cured
3	Sepsis & meningitis (Neisseria meningitidis)	42	f	0.15	5.27	0.07	5.7	Cured
4	Sepsis & meningitis (Strep. pneu.)	56	f	0.59	15.00	0.1	5.6	Deceased
5	Sepsis & encephalitis & endocarditis (coagulase-negative Staph.)	84	f	1.97	39.64	0.18	10.4	Deceased*
6	Sepsis (E. coli)	67	f	0.61	42.28	0.16	5.7	Deceased
7	Sepsis (Neisseria meningitidis)	33	f	2.31	76.14	0.08	5.1	Deceased
8	Sepsis	66	m	0.62	16.82	0.13	3.88	Deceased
9	Sepsis & meningitis (Strep. pneu.)	37	m	0.19	16.39	0.11	5.75	Cured
10	Sepsis (Staph. aureus)	70	m	0.81	21.9	0.15	6.48	Deceased
11	Sepsis & meningitis (Neisseria meningitidis)	46	m	0.18	5.26	0.13	5.34	Cured
12	Sepsis (coagulase-negative Staph.)	75	m	0.19	15.01	0.18	11.3	Cured
13	Sepsis	62	m	0.43	9.97	0.17	10.84	Deceased
14	Sepsis & pneumonia	76	m	0.28	12.47	0.18	9.36	Cured
15	Sepsis (Staph. epidermidis)	71	m	0.28	14.88	0.17	7.71	Cured
Median				0.59	15	0.15	5.75	

Staph., *Staphylococcus*; Strep., *Streptococcus*; pneu., *pneumoniae*; E. coli, *Escherichia coli*; m, male; f, female.

Control vs septicemia: $P = 0.004$ for activin concentrations and $P = 0.009$ for FS concentrations (paired Wilcoxon rank test).

* Patient died soon after discharge from clinic.

Figure 1 Time course of activin, FS and CRP concentrations in serum of female (A) and male (B) patients with septicemia. Patient numbers correspond to the numbers in Table 1. In each diagram on the X-axis, the time points of blood sampling are shown (first sample taken at 0 h). On the left Y-axis, FS serum concentrations in ng/ml and on the right Y-axis activin serum concentrations in ng/ml are shown. The second Y-axis on the left is the scale for the CRP serum concentrations in ng/ml.

(B) patients and the corresponding serum levels of CRP, an indicator of inflammation. Most of the diagrams in Fig. 1 show that activin and FS serum levels track each other and follow changes in CRP levels. Overall, activin and FS concentrations were correlated with each other ($r^2 = 0.64$). There was no apparent relationship between activin and FS serum concentrations and the number of leukocytes ($r^2 = 0.09$). The parallel profiles of FS, activin, and CRP suggest a causal relationship between bacterial infection and elevated activin and FS serum levels. The observed increases in FS and activin serum concentrations during the inflammatory response are in accordance with observations in animal experiments, where interleukin-1 β or lipopolysaccharide (LPS) injections caused significantly elevated FS and activin serum levels (23, 37, 38). The onset of septicemia is often unnoticed, whereas in most cases blood sampling commenced with obvious signs of the disease; this made it impossible to determine whether the serum concentration of activin, FS or CRP was the first to rise at the beginning of the infection.

The normal creatinine serum concentrations in most patients and the molecular size of the glycosylated FS (39–45 kDa) and activin (25 kDa) molecules make an altered renal function as the sole cause of increased serum concentrations in sepsis unlikely. The tissues or cell types which contribute to the activin and FS serum levels in normal and infected animals or humans are currently unknown. Leukocyte counts did not show a strong correlation with FS and activin levels in septicemic patients ($r^2 = 0.09$) and therefore leukocytes may not be the primary source of increased FS and activin levels during septicemia. Nevertheless, the lack of correlation with total leukocyte counts does not rule out the possibility that specific populations of cells preferentially release activin and/or FS, as has been documented for human monocytes in response to LPS (28, 39).

Well-balanced steady-state levels of FS and activin govern the growth, differentiation and behavior of many cell types (see Introduction), and pharmacological doses of FS and activin affect pituitary hormone secretion in animals (17, 40–48). Therefore, it is conceivable that elevated FS and activin serum levels can also influence endocrine hormone patterns or paracrine interactions in tissues. Elevated serum concentrations of activin and/or FS have been reported in a number of pathological states, including hypertension during pregnancy, renal failure, liver dysfunction and various carcinomas (16, 49–54). Nevertheless, the role of these proteins in the disease process is poorly defined. The need for a well-balanced equilibrium of FS and activin could be one explanation for the tight correlation of both factors in serum during sepsis ($r^2 = 0.64$). In terms of inflammatory processes, emerging data suggest activin can have both pro- and anti-inflammatory effects on cell responses and cytokine

production, which may account for its elevated levels in patients with septicemia. Nevertheless, these studies have focused largely on cell cultures of primary immune cells or cell lines. Therefore, the pathophysiological relevance of release of activin and FS to septicemia remains to be elucidated.

In conclusion, activin and FS serum concentrations are elevated in patients with septicemia. The rises are accompanied by increased serum CRP levels. Detailed studies are necessary to determine the cell type(s) responsible for the FS and activin increases in the serum of septicemic individuals and to elucidate the pathophysiological relevance of this phenomenon.

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