Original Contributions

INTERLEUKIN-6 ADMINISTRATION HAS NO ACUTE HEMODYNAMIC OR HEMATOLOGIC EFFECT IN THE DOG

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To investigate the possible hemodynamic effects of interleukin-6 (IL-6), a single dose of 15 mcg/kg of recombinant IL-6 isolated from *Escherichia coli* was injected intravenously in six pentobarbital-anesthetized dogs. After 30 min, saline infusion was performed to maintain the pulmonary artery balloon-occluded pressure at baseline level. The animals were observed for up to 5 hours. No other hemodynamic alteration was observed than a gradual decline in cardiac output attributed to anesthesia. Hematologic variables, blood glucose, and total serum proteins were also constant. IL-6 levels were markedly elevated in the blood, but no tumor necrosis factor activity was detected. Thus a primary role for IL-6 in the early cardiovascular alterations associated with septic shock seems unlikely.

Various cytokines have been implicated in the development of the hemodynamic alterations characterizing severe sepsis. In particular tumor necrosis factor (TNF), interleukin-1 (IL-1), and interleukin-2 (IL-2) are released during severe infections or following intravenous challenge with endotoxin. If given by themselves, they have been shown to produce the decrease in arterial pressure, the increase in cardiac output, and the fall in systemic vascular resistance that characterize sepsis.[1-5]

Interleukin-6 (IL-6) is another mediator released by various cells under the influence of other cytokines like TNF and IL-1, bacterial products like endotoxin, and even viruses.[6-8] IL-6 has various metabolic and immunological effects, including the release of many metabolically active substances from the liver. In man, IL-6 is released following administration of endotoxin[8] or TNF.[9] An association between IL-6 levels and fatal outcome has been found in patients with septic shock (references 10 and 11, and M.R. Pinsky et al., unpublished data).

However, it is not known whether IL-6 participates in the hemodynamic disturbances associated with septic shock. Therefore, the present study evaluated the acute effects of IL-6 administration in a dog model.

RESULTS

The effects of IL-6 on the measured and derived hemodynamic variables are summarized in the Table 1. Intravenous fluid requirements were very limited to maintain the end-expiratory PA balloon-occluded pressure (Ppao) at baseline levels (mean 7.1 ± 12.5 ml/kg/h). Throughout the study, IL-6 administration had no hemodynamic effect other than a gradual decrease in cardiac output and a corresponding increase in systemic vascular resistance. After a transient decline, the core temperature tended to rise during the last 2 h of the experiment. There was a small and non-significant increase in VO_2 and O_2 extraction, as well as in white blood count (WBC). Blood glucose did not change.

Serum IL-6 levels increased markedly in all animals but showed marked interanimal variability in levels (Table 2). No TNF activity was detected in the blood.

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Table 1. Hemodynamic and metabolic effects of IL-6 administration in the 6 dogs.

Variable	Time (min)						
	0 (Baseline)	30	60	120	180	240	300 (4 dogs)
Blood temp, °C	37.3 ± 1.2	37.3 ± 1.2	37.3 ± 1.3	37.1 ± 1.4	37.1 ± 1.4	37.4 ± 1.4	37.9 ± 1.0
Heart rate, bpm	128.2 ± 11.7	145.0 ± 11.5	137.0 ± 7.9	138.3 ± 14.6	127.0 ± 28.4	138.0 ± 30.8	147.5 ± 34.2
Mean Art. P., mmHg	114.4 ± 26.7	106.9 ± 25.7	114.4 ± 25.6	119.9 ± 8.5	124.4 ± 8.6	122.4 ± 15.1	117.1 ± 17.3
Pulm. Art. P., mmHg	11.1 ± 2.0	11.6 ± 0.9	11.4 ± 1.3	11.8 ± 0.9	13.4 ± 4.1	12.9 ± 0.8	13.6 ± 2.0
Pulm. Art. Balloon-occl P., mmHg	4.0 ± 0.8	5.4 ± 1.5	4.5 ± 1.0	5.0 ± 1.2	5.3 ± 1.3	5.7 ± 0.8	5.8 ± 0.8
Right Atrial P., mmHg	2.5 ± 1.0	2.8 ± 1.3	2.7 ± 1.4	3.3 ± 1.1	2.8 ± 0.7	3.0 ± 1.2	2.8 ± 0.4
Cardiac output, L/min	6.0 ± 2.4	6.3 ± 1.4	5.3 ± 1.1	3.9 ± 0.5	3.5 ± 1.8	2.9* ± 0.9	3.5 ± 1.0
Syst. Vasc. Res, dynes sec cm^{-5}	1692 ± 641	1342 ± 205	1690 ± 190	2393 ± 261	2900* ± 687	3555† ± 1196	2686 ± 475
Pulm. Vasc. Res, dynes sec cm^{-5}	113.8 ± 54.3	85.7 ± 35.3	109.7 ± 31.8	142.0 ± 33.6	190.0 ± 58.1	216.6 ± 75.2	186.5 ± 38.9
Left Ventr. Stroke Work, g.m.	72.3 ± 34.2	61.9 ± 22.8	60.2 ± 23.3	45.7 ± 12.1	47.6 ± 17.3	36.9 ± 20.8	42.4 ± 28.1
Right Ventr. Stroke Work, g.m.	5.6 ± 2.6	5.2 ± 1.6	4.7 ± 1.5	3.3 ± 0.7	4.2 ± 1.8	3.0 ± 1.3	4.0 ± 2.4
PaO_2, mmHg	90.2 ± 0.2	92.1 ± 0.2	90.8 ± 0.2	95.4 ± 0.2	95.8 ± 0.1	93.3 ± 0.1	84.4 ± 0.1
Hemoglobin conc., g/dl	12.0 ± 1.1	12.4 ± 0.7	12.7 ± 1.6	12.7 ± 0.9	12.6 ± 1.3	12.8 ± 1.4	12.5 ± 1.6
O_2 Transport, ml/min	883 ± 340	984 ± 258	842 ± 221	631 ± 103	554 ± 123	468 ± 130	538 ± 148
O_2 Consumption, ml/min	164 ± 48	221 ± 86	218 ± 80	273 ± 128	291 ± 121	336 ± 99	329 ± 114
O_2 Extraction, %	0.28 ± 0.09	0.26 ± 0.08	0.30 ± 0.09	0.33 ± 0.09	0.34 ± 0.09	0.36 ± 0.06	0.36 ± 0.07
Blood Lactate, mEq/L	1.34 ± 0.82	1.22 ± 0.67	1.23 ± 0.60	1.08 ± 0.51	1.22 ± 0.58	1.10 ± 0.57	0.95 ± 0.55
Glucose, mg/dl	173.0 ± 9.6	178.0 ± 21.5	178.5 ± 29.3	171.2 ± 18.8	161.7 ± 9.4	171.2 ± 8.4	180.8 ± 20.1
Total protein, g/dl	7.50 ± 0.89	7.50 ± 0.91	7.58 ± 0.82	7.12 ± 0.71	7.15 ± 0.52	7.10 ± 0.44	7.15 ± 0.41
Hematocrit, %	34.6 ± 2.8	35.3 ± 2.1	36.6 ± 4.4	36.8 ± 2.5	36.9 ± 3.5	37.5 ± 3.8	37.9 ± 5.7
White blood cells/mm^3	3.7 ± 3.1	3.8 ± 3.3	4.8 ± 2.2	7.0 ± 5.2	6.2 ± 3.6	9.6 ± 6.1	11.2 ± 5.9
Platelets/mm^3	206 ± 45	183 ± 81	173 ± 78	190 ± 43	150 ± 64	217 ± 27	207 ± 29

*$p < 0.05$.
†$p < 0.01$.

DISCUSSION

Severe sepsis can trigger the release of various inflammatory and immunological substances into the circulation, including peptides (bradykinin, histamine), eicosanoid metabolites (prostacyclin, thromboxane A_2, prostaglandin E_2), and cytokines (TNF, IL-1, IL-2), all of which have been shown to either contribute to or mimic specific aspects of the septic hemodynamic response.[1-5] IL-6 is another cytokine that is also released into the circulation early in the course of human sepsis[12,13] and may be involved in the resultant inflammatory and metabolic response. IL-6 stimulates hematopoiesis and production and release of acute phase proteins,[14] and may stimulate adrenocorticotropic hormone (ACTH) release and insulin resistance. However, the acute hemodynamic and hematologic effects of IL-6 are unknown. Based on the findings of this study, however, IL-6 has no significant acute hemodynamic or hematologic effects in the dog when given alone as a bolus. We did see a gradual and progressive decrease in cardiac output and an increase in systemic vascular resistance. This effect was most likely due to pentobarbital anesthesia. Furthermore, in the fluid resuscitated dog model, sepsis and sepsis-like syndromes are associated with an increase in cardiac output and a decrease in systemic vascular resistance.

We can think of several possible reasons for our inability to observe any hemodynamic or hematologic effects of IL-6 in the dog. First, the amount of IL-6 may

Table 2. Serum IL-6 levels (in U/ml) in the 6 dogs.

Dog number	Time (min)						
	0 (Baseline)	30	60	120	180	240	300
1	13	5,368	3,391	690	115	50	26
2	46	11,456	4,710	6,969	2,223	1,552	—
3	28	1,677	640	298	99	63	—
4	19	1,728	802	391	156	72	26
5	5	304	165	62	26	15	13
6	15	597	72	17	14	13	5
Mean	21	3,522	1,630	1,405	439	294	18
± SD	14	4,287	1,940	2,737	876	617	10

have been inadequate to induce any measurable effect, although larger doses would have. This seems unlikely since the dosage level was chosen has been shown to induce metabolic effects in the rat,[15] and IL-6 blood levels were similar to those measured by us in the dog following endotoxin administration (5 mcg/kg) (unpublished data). So these levels seem appropriate. Second, the specific IL-6 used in this study may not be active in the dog. Species variability to susceptibility to endotoxin and cytokines is well known. However, IL-6 is a small oligopeptide and does induce metabolic changes in a variety of animal species. Nevertheless, we cannot entirely exclude this possibility. Third, since we only noted the effects of IL-6 for the first 5 h following its infusion, if the metabolic effects of IL-6 determine any related hemodynamic ones, then we would not see them. However, it would be difficult to have an acute canine model of IL-6 infusion stable for longer periods of instrumentation. Furthermore, IL-6 release occurs early in the course of human septic shock,[12,13] usually within the first hour. If delayed actions do occur, it is unlikely that IL-6 is a primary mediator of the acute hemodynamic response to sepsis, but our data do not exclude the possibility that IL-6 is important in the overall response to established sepsis. Fourth, IL-6 may require the interaction of other cytokines to be active. This seems reasonable, since it requires TNF and IL-1 for its release.[9,10] Although clearly released during sepsis in association with other cytokines, and thus potentially requiring such cytokine interactions to be active, other cytokines, such as TNF, IL-1, and IL-2, have significant hemodynamic effects if given alone.

IL-6 may exert systemic effects not measured in the present study. IL-6 may function more as an immunoregulating cytokine through the production of acute phase reaction proteins and cortisol release. These important metabolic regulatory effects of IL-6 may occur during the acute phases of shock but might not alter any of the observed variables characterizing septic shock. Accordingly, on the basis of this study, a primary role of IL-6 in initiating the early hemodynamic alterations associated with septic shock is unlikely.

MATERIAL AND METHODS

Six large mongrel dogs (22 ± 4 kg) were anesthetized with pentobarbital sodium (25 mg/kg IV, followed by a constant infusion of 4 mg/kg/h), and their tracheas intubated and ventilated with room air. The details of the experimental setting have been previously described.[16-18] Briefly, the animal was instrumented with arterial and venous catheters, and a pulmonary artery (PA) balloon-tipped catheter to allow measurements of arterial, right atrial, and PA pressures and cardiac output by thermodilution and arterial and mixed venous blood gases.

After baseline measurements following instrumentation, a single dose of 15 mcg/kg of IL-6 was administered through the central venous catheter. Recombinant human IL-6 of full biological activity was isolated from *Escherichia coli.*[19] The concentration was 6.6 mg protein/ml 50% acetonitrol. (IL-6 was provided by W. Sebald). This dose of IL-6 has been shown to induce metabolic effects in previous animal experiments.[15] Thirty minutes following the infusions of IL-6, saline infusion was started to maintain an end-expiratory PA balloon-occluded pressure (Ppao) at baseline level. There was no attempt to control the temperature of the animal. Hemodynamic measurements and blood sampling were performed at baseline, after 30 min, 1, 2, 3, 4 (all dogs), and 5 h (4 dogs). Derived hemodynamic variables were calculated by standard formulae.[16-18] Appropriate blood samples were assayed for arterial and mixed venous blood gases (IL 282 Radiometer, Copenhagen), hemoglobin and hematocrit, white blood count (WBC), platelet count, blood lactate (enzymatic method), glucose (Dextrostix), and total proteins (refractometry). Blood samples were also collected simultaneously in glass pyrogen-free tubes and stored at −70°C prior to measurement of serum IL-6 and TNF levels. Serum IL-6 activity was assayed using an IL-6–dependent mouse hybridoma, 7TD1, cultivated in flat-bottomed microtiter plates containing 2,000 cells per well in the presence of serial dilutions of the serum sample. After 4 days of culture, the number of surviving cells was determined by a colorimetric assay for hexosaminidase, as previously described.[20] Hybridoma growth factor/IL-6 activity was expressed in U/ml defined as the dilution giving half maximal proliferation of 7TD1 cells. One unit corresponds to approximately 5 pg/ml of IL-6. 7TD1 cells do not respond to TNF, IL-1β, IL-2, interferon α, β, or γ, nor to any of the known colony-stimulating factors other than IL-6.[20] The biological activity of IL-6 samples was completely neutralized by adding monospecific rabbit polyclonal anti-recombinant human IL-6 antibodies to the test samples. The inter-assay variability (±30%) was corrected by the use of an internal standard. Each sample was tested at least four times. To eliminate inhibitory effects present in undiluted sera the samples were bathed at 56°C for 30 min before the assay (J. Content).

TNF levels were determined by a modified TNF-specific ELISA technique (W. Buurman).

Statistical analysis was performed using an analysis of variance for repeated measures and a post-hoc Student-Newman-Keuls test. An f ratio corresponding to a p value less than 0.05 was considered statistically significant.

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