Model of septic shock induced by live *E. coli* (O18) in a laboratory rat

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ABSTRACT: This study was concerned with the development of induced septic shock in a laboratory rat using a series of measurements including body temperature, heart and respiratory rates, haematocrit value, red and white blood cell counts, differential leukocyte count, haemoglobin value, glycaemia, analysis of arterial blood gases, and serum levels of interleukin 6 (IL-6) during the first five hours. A total of 12 specific pathogen free (SPF) laboratory rats were used for the study. Septic shock was induced under general anaesthesia by introducing live *E. coli* (O18) into the jugular vein in the dose of 1×10^9 per 100 g of body weight (group SESH). Clinical measurements and blood collection from *a. carotis* were performed just prior to, and then 1.5 and 5 h after the administration of *E. coli*. The control group (C) contained 9 SPF laboratory rats which received physiological saline only, at the same volume into the jugular vein, and blood collection followed according to the same scheme as above described for group SESH. The results of the experiment showed that changes in clinical, haematological and biochemical parameters could be detected as early as 1.5 hours after induction. These changes correspond with the activation of an inflammatory reaction and the development of metabolic acidosis. They are accompanied by a considerable rise in IL-6 already 1.5 h after the application of live *E. coli* and after 5 h the levels exceeded 2 000 pg/ml in all experimental animals. Our results clearly document the importance of IL-6 for the early detection of developing septic shock and of some less specific but routinely determined parameters such as white blood cell count and base excess.

Keywords: sepsis; interleukin 6; systemic inflammatory response syndrome

Despite advances in critical care, in human medicine sepsis and septic shock due to infection play a considerable role in the morbidity and mortality of patients of intensive care units (Martin et al., 2003). According to several published studies and because of the similarity of these processes, high levels of morbidity and mortality may also be expected in veterinary medicine (Vela et al., 2006).

Micro-organisms proliferate following an infection and produce endotoxins and exotoxins which stimulate the primary immune system, and endothelial and other cells. The nuclear factor κB (NF-κB) is activated, followed later by the production of the macrophage migration inhibiting factor (MIF), tumour necrotizing factor-α (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6), free oxygen radical and reactive nitrogen species. The activation of the above factors results in the induction of systemic inflammatory reactions and myocardial depression observed during sepsis. In serious cases this may lead to multi-organ dysfunction (MODS), or even multi-organ failure (MOF) (Das, 2003).

Uncontrolled, inadequate or late resuscitation and a failure of compensatory mechanisms may lead to tissue hypoxia, anaerobic glycolysis and a disturbance in the acid-base balance. There is,

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therefore, a demand for reliable biochemical or immunological markers differentiating individual kinds of sepsis and helping in the selection of therapeutic strategies. Parameters important for the patient from the prognostic and pathogenetic point of view include, apart from others, cytokines – IL-1, IL-6, TNF-α and acute phase reactants (C-reactive protein, etc). IL-6 is a protein produced *in vivo* by activated monocytes, fibroblasts and endothelial cells, in particular. It is considered a cytokine with pleiotropic effects which plays an important role in the induction and control of cellular reactions of the acute phase and is an important mediator in the development of shock and multi-organ failure (Johnson et al., 2004).

Monitoring of serum levels of early mediators of inflammation such as IL-1, IL-6 or TNF-α may be a effective method of evaluating the inflammatory response and in the early detection of acute phase reactions allowing the determination of the most appropriate therapeutic approach (Hack et al., 1989; Borden and Chin, 1994; Liaw et al., 1997; Harbath et al., 2001). At the same time, the levels of inflammatory mediators or the duration of their rise correlate well with the seriousness of the state of critical care patients and evidently have a prognostic role (Pinsky et al., 1993; Martin et al., 1994, 1997; Taniguchi et al., 2003).

This study was aimed at monitoring the dynamics of the development of septic shock using some clinical, haematological and biochemical parameters in correlation with serum levels of IL-6, an important mediator of the immune response, under conditions accurately simulating clinical reality following the intravenous administration of live bacteria and subsequent bacteriemia.

MATERIAL AND METHODS

A total of 21 laboratory rats (outbred specific pathogen free Wistar, AnLab Prague) weighing 545 g on average (min. 420 g; max. 545 g) were used for the study and treated in accordance with the Regulation 86/6091 EEC and the Project approved by the Ethical Committee of the University of Veterinary and Pharmaceutical Sciences Brno.

Animals were divided into two groups, i.e. septic shock group (SESH, n = 12) and control group (C, n = 9). Prior to the experiment, the animals were examined so as to check their state of health, body weight, temperature, respiratory and heart rates. After that, general anaesthesia was induced in the experimental animals using atropine (0.05 mg/kg body weight), droperidol (0.8 mg/kg body weight), ketamine (35 mg/kg body weight) and xylazine (5 mg/kg body weight). When necessary, a prolongation of anaesthesia during the experiment was effected by administering half the dose of ketamine (Scheer et al., 2003). Under general anaesthesia and after the preparation of *a. carotis* and *v. jugularis*, intravenous catheters were inserted into these vessels to enable blood collection and intravenous administration of drugs. The animals were placed in dorsal recumbency on a non-heated pad in a room with a constant temperature of 22 ± 2°C.

Time 0

After inducing general anaesthesia and making the vascular bed accessible, the rectal temperature was measured using a digital thermometer with a range of 28.0–42.9°C (Afec T04); respiratory rates and pulse rates were measured using a pulsemeter (Nonin 8500AV, transflex probe 2000T, arithmetical mean from three subsequent measurements). A sample of arterial blood (2 ml) was taken from a catheter inserted into *a. carotis* for laboratory examination and replaced by the same volume of physiological saline in both groups of animals. After that, *v. jugularis* was used for administration of live *E. coli* bacteria (O18) in the dose of 1×10^9/100g of body weight to animals of group SS (Solution concentration: 1×10^9 *E.coli* (O18)/ml) (Tanaka et al., 1983). Group C animals received an equivalent volume of physiological saline.

Time 1.5 h and 5 h

1.5 h and 5 h after the intravenous administration of live *E. coli* bacteria to group SESH or administration of physiological saline to group C parameters such as body temperature, heart and respiratory rates were determined. At the same time, 2 ml of arterial blood were withdrawn and replaced by the same volume of physiological saline in both groups.

Sample processing

Immediately after collection, arterial blood glucose was determined using a glucometer with

a measurable range from 1.1 to 33.4 mmol/l (Glucocard II, Arkray). Standard haematological parameters such as haematocrit value, red and white blood cell counts and the differential leukocyte count were determined manually. Arterial blood gases and levels of essential ions including pH, pCO_2, HCO_3^-, p_aO_2, BE, and the anion gap were also examined (Rapidlab 855, Bayer Diagnostics). A part of the sample was used to produce serum which was immediately frozen and stored at –20°C until the IL-6 analysis. Serum concentrations of IL-6 were evaluated following thawing by the ELISA method using a commercially available assay (IL-6 (rat) EIA, DRG Diagnostics, sensitivity from 8 to 2 000 pg/ml) in the laboratory of the Department of Microbiology and Immunology of the University of Veterinary and Pharmaceutical Sciences, Brno. The samples in which IL-6 concentrations exceeded 2 000 pg/ml were diluted and re-evaluated.

Statistical data evaluation

Statistical analysis was based on all obtained results, including those from animals with incomplete data due to the impossibility of analysis (a lack of samples from those animal who died during the experiment). Nevertheless, data on each parameter was made up of at least six values.

Considering both groups of animals, arithmetical means and standard deviations were computed for individual parameters. Data were then evaluated using the *F*-test and, based on that, the *t*-test parameters were selected. The IL-6 concentration values were evaluated using Horn's parallel analysis to predict the 95th percentile point in distributions of eigenvalues generated from random data matrices and the results were graphically expressed. Undetectable levels of IL-6 (less than 8 pg/ml) were considered to be 0 for purposes of the statistical analyses.

Commercially available software was used for the statistical analysis, i.e., SPSS and Microsoft Office Excel 2003.

RESULTS

It was not possible to collect samples and perform clinical measurements at the 5 h interval in three out of 12 animals in which septic shock was induced (group SESH) because of their death prior to the termination of the experiment (25% mortality). Table 1 to Table 4 show the results of obtained parameters including the arithmetical mean and standard deviation as well as the results of evaluation within the group using the paired Student's *t*-test and in comparison with the control group using the non-paired Student's *t*-test for individual parameters.

Evaluation of the development of measured parameters within the group

The following results were obtained upon the statistical evaluation of values of measured parameters at individual times in the group where septic shock was experimentally induced.

In the monitored group there was a statistically highly significant drop in temperature between measurements at 0 h and 1.5 h ($P = 0.002$); when compared with time 0 the temperature was also considerably lower at time 5 h ($P = 0.003$). The pulse rate did not change significantly between time 0 h and 1.5 h; values at time 5 h, however, were significantly lower than at time 0 h ($P < 0.001$). Respiratory rates at any time were not different from values at time 0 h. There were no statistical differences between measurements in values of glycaemia. Values of IL-6 at time 1.5 h were significantly higher compared with time 0 h ($P = 0.001$) and levels of IL-6 at time 5 h also significantly increased compared with values at time 0 h ($P < 0.001$) – see Table 1.

No significant differences were observed in haematocrit values between time 0 h and 1.5 h but values at time 5 h were significantly lower compared to time 0 h ($P < 0.001$). Similar changes were noted in red blood cell counts. White blood cell count dropped significantly between measurements at time 0 h and 1.5 h ($P = 0.008$); at time 5 h white blood cells amounted to a significantly lower value compared with time time 0 h ($P < 0.001$). Neutrophil band counts at time 5 h compared with time 0 h were significantly increased ($P = 0.028$). Segmented neutrophil counts at time 1.5 h were similar to those at time 0 h; counts at time 5 h, however, significantly increased compared with time 0 h ($P = 0.002$). Band and segmented neutrophils increased at the expense of lymphocyte counts, which were not considerably altered at time 1.5 h compared with time 0 h, while counts at time 5 h were with a high statistical significance lower than at time 0 h ($P = 0.006$) – see Table 2.

Table 1. Septic shock group – triage, glycaemia, interleukin 6

	Temperature (°C)			Pulse (n/min)			Respiration (n/min)			Glukose (mmol/l)			IL-6 (pg/ml)		
Hour	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5
Arithmetic mean	33.5	30.2	29.6	251.8	245.3	115.3	61.3	72.5	52.6	19.5	19.7	19.8	4.6	306	2419
Standard deviation	1.4	2.8	2.9	49.9	93.4	21.6	10.3	22.9	9.4	4.6	8.5	12.2	15.9	251	270
Pair *t*-test (*P*)	0.002*[a]	0.003*[b]	–	0.819[a]	< 0.001*[b]	–	0.169[a]	0.16[b]	–	0.881[a]	0.849[b]	–	0.001*[a]	< 0.001*[b]	–
t-test with controls (*P*)	0.063	0.083	0.090	0.117	0.584	< 0.001*	0.246	0.665	0.001*	0.564	0.299	0.127	0.273	0.003*	< 0.001*

*$P < 0.05$; [a]*P*-value of pair *t*-test of values at 0 vs. 1.5 h; [b]*P*-value of pair *t*-test of values at 0 vs. 5 h

Table 2. Septic shock group – haematology

	Haematocrit (l/l)			Erythrocytes (× 10)			Leucocytes (×10^9)			Bands			Segments			Lymphocytes		
Hour	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5
Arithmetic mean	0.40	0.38	0.36	7.19	6.52	6.4	11.27	9.27	3.10	0.00	0.02	0.04	0.17	0.21	0.30	0.81	0.76	0.67
Standard deviation	0.03	0.05	0.04	1.83	1.36	0.77	4.58	4.28	1.19	0.01	0.02	0.04	0.09	0.07	0.11	0.09	0.07	0.11
Pair *t*-test (*P*)	0.079[a]	< 0.001*[b]	–	0.068[a]	0.007*[b]	–	0.008*[a]	< 0.001*[b]	–	0.025*[a]	0.028*[b]	–	0.221[a]	0.002*[b]	–	0.231[a]	0.006*[b]	–
t-test with controls (*P*))	0.830	0.737	0.059	0.821	0.680	0.234	0.825	0.299	< 0.001*	0.655	0.049*	0.040*	0.440	0.792	0.058	0.580	0.815	0.125

*$P < 0.05$; [a]*P*-value of pair *t*-test of values at 0 vs. 1.5 h; [b]*P*-value of pair *t*-test of values at 0 vs. 5 h

Table 3. Septic shock group – blood gases

	pH			p_aCO_2 (kPa)			p_aO_2 (kPa)			HCO^-_3 (mmol/l)			Base excess (mmol/l)			S_aO_2 (%)			$p_{A\text{-}a}O_2$ (kPa)		
Hour	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5
Arithmetic mean	7.47	7.48	7.32	5.22	4.66	4.26	8.73	9.65	11.05	27.6	25.8	16.8	3.2	1.5	–9.8	97.2	97.6	93.7	4.91	4.86	3.87
Standard deviation	0.06	0.05	0.16	0.84	0.82	1.31	1.25	2.59	2.61	1.5	1.7	4.0	2.0	1.9	5.4	4.4	5.1	13.2	1.57	2.42	2.66
Pair *t*-test (*P*)	0.660[a]	0.047*[b]	–	0.169[a]	0.057[b]	–	0.302[a]	0.034*[b]	–	0.013*[a]	< 0.001*[b]	–	0.061[a]	< 0.001*[b]	–	0.658[a]	0.560[b]	–	0.950[a]	0.444[b]	–
t-test with controls (*P*))	0.068	0.380	0.036*	0.440	0.682	0.705	0.385	0.202	0.418	0.410	0.445	< 0.001*	0.657	0.470	0.001*	0.190	0.371	0.254	0.071	0.113	0.212

*$P < 0.05$; [a]*P*-value of pair *t*-test of values at 0 vs. 1.5 h; [b]*P*-value of pair *t*-test of values at 0 vs. 5 h

pH values in the septic shock group were not significantly different between time 0 h and 1.5 h; but there was a significant drop between time 0 h and 5 h ($P = 0.047$). No significant changes in p_aCO_2 were found during the experiment. Changes in values of p_aO_2 between time 1.5 h and 0 h were not statistically significant; nevertheless, values at time 5 h were significantly higher than at time 0 h ($P = 0.034$). There was a considerable drop in HCO_3^- values after the lapse of 1.5 h from the start of the experiment ($P = 0.013$); this parameter also dropped significantly between time 0 h and 5 h ($P < 0.001$). Values of BE did not change considerably up to 1.5 h from the start of the experiment; however, at time 5 h, they were significantly lower when compared with the initial value at time 0 h ($P < 0.001$). No statistically significant changes were observed in values of SaO_2 and $p_{A\text{-}a}O_2$ – see Table 3.

Levels of sodium (Na^+) between time 0 h did not differ significantly; statistical differences were found, however, when comparing lower values at time 5 h with those at time 0 h ($P = 0.049$). Levels of potassium (K^+) at 1.5 h from the start of the experiment had increased significantly ($P = 0.019$); there was, however, no difference at time 5 h when compared with time 0 h. No significant changes were found in levels of ionized calcium (Ca^{++}) and chlorides (Cl^-). To begin with, anion gap values did not change significantly; however, between time 0 h and 5 h there was a significant increase ($P < 0.001$) – see Table 4.

Evaluation of the development of measured parameters compared with the control group

The following results were obtained after statistical comparison of the values of measured parameters between the experimental and control group:

There were no statistical differences in temperature at any time of measuring. Pulse rates in the septic shock group at time 0 h and 1.5 h were not significantly different from the control group while at time 5 h it was significantly lower ($P < 0.001$). Similarly, respiratory rates in the septic shock group at time 0 h and 1.5 h were not significantly different from the control group while at time 5 h it was significantly lower ($P < 0.001$). No significant differences were found between the septic shock group and the control group at any time of measurement. Levels of IL-6 at time 0 h did not differ significantly; however, they were significantly higher at time 1.5 h ($P = 0.003$) and even more significantly higher at time 5 h when compared with the control ($P < 0.001$) – see Tables 1 and 5.

Comparison of the septic shock group and the control group with regard to values of haematocrit and red blood cell count revealed no differences. A considerable decrease in white blood cell count ($P < 0.001$) was observed in the septic shock group at time 5 h, while in earlier measurements no differences from the control were found. The evaluation of differential counts of band neutrophils uncovered no differences at time 0 h, while the values increased at time 1.5 h and 5 h ($P = 0.049$; $P = 0.040$). No differences were found in counts of segmented neutrophils and lymphocytes at any time of measurement – see Tables 2 and 6.

There were no statistically significant differences in pH values in the septic shock group compared to the control group at time 0 h and 1.5 h; at time 5 h the pH value was significantly lower ($P = 0.036$). Differences in values of p_aCO_2 and p_aO_2 were insignificant at each time of measurement. Values of HCO_3^- showed no differences at time 0 and 1.5 h but decreased with a high statistical significance in the septic shock group at time 5 h ($P < 0.001$). Similarly, values of base excess showed no differences at time 0 and 1.5 h but decreased statistically significanctly in the septic shock group at time 5 h ($P < 0.001$). No differences were found in values of SaO_2 and $p_{A\text{-}a}O_2$ at any time of measurement – see Tables 3 and 7.

The evaluation of levels of sodium (Na^+), potassium (K^+), ionized calcium (ioniz. $Ca2^+$) and chlorides (Cl^-) did not succeed in identifying any significant differences. Values of anion gap (AnGap) showed no differences at time 0 and 1.5 h but were statistically significantly elevated in the septic shock group at time 5 h ($P = 0.008$) – see Tables 4 and 8 .

Figure 1 shows the area of distribution of IL-6 levels in the septic shock group and the control group in 95% probability and is bound by pivots $L_{D,95}$ (lower pivot in 95th percentile) and $L_{H,95}$ (higher pivot in 95th percentile); a curve PL is plotted through the semisums of the pivots. A pronounced increase in IL-6 levels is evident after 1.5 h in the septic shock group, whereas the IL-6 levels in the control group did not exceed the detection level (8 pg/ml) during the whole study. The levels of IL-6 further increased in the septic shock group at 5 h after the initiation of the experiment.

Table 4. Septic shock group – ions

	Na^+ (mmol/l)			K^+ (mmol/l)			Ionized Ca^{2+} (mmol/l)			Cl^- (mmol/l)			AnGap		
Hour	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5
Arithmetic mean	136.1	129.5	134	4.42	4.74	4.93	1.30	1.19	1.30	103.3	103.7	104.0	7.9	7.4	17.4
Standard deviation	2.0	12.7	2.9	0.43	0.48	1.34	0.05	0.38	0.10	3.0	5.4	5.7	3.2	4.3	7.6
Pair *t*-test (*P*)	0.094[a]	0.049*[b]	–	0.019*[a]	0.247[b]	–	0.349[a]	0.543[b]	–	0.756[a]	0.766[b]	–	0.785[a]	0.002*[b]	–
t-test with controls (*P*)	0.151	0.099	0.054	0.861	0.635	0.093	0.563	0.087	0.301	0.844	0.353	0.188	0.079	0.299	0.008*

*$P < 0.05$; [a]*P*-value of pair *t*-test of values at 0 vs. 1.5 h; [b]*P*-value of pair *t*-test of values at 0 vs. 5 h

Table 5. Control group – triage, glycaemia, interleukin 6

	Temperature (°C)			Pulse (n/min)			Respiration (n/min)			Glukose (mmol/l)			IL-6 (pg/ml)		
Hour	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5
Arithmetic mean	34.6	32.0	31.4	284.4	265.1	233.8	56.7	68.7	71.8	18.3	16.4	12.9	55.6[‡]	0[‡]	0[‡]
Standard deviation	1.1	0.6	1.0	37.5	58.6	71.4	5.8	14.2	10.4	4.5	4.2	4.1	157.3[‡]	0[‡]	0[‡]
Pair *t*-test (*P*)	< 0.001*[a]	< 0.001*[b]	–	0.380[a]	0.093[b]	–	0.049*[a]	0.008*[b]	–	0.056[a]	0.003*[b]	–	0.351[a]	0.351[b]	–

*$P < 0.05$; [a]*P*-value of pair *t*-test of values at 0 vs. 1.5 h; [b]*P*-value of pair *t*-test of values at 0 vs. 5 h; [‡]enter data under detectable level of 8 pg/ml were considered 0 for purposes of analysis

Table 6. Control group – haematology

	Haematocrit (l/l)			Erythrocytes ($\times 10^{12}$)			Leucocytes ($\times 10^9$)			Bands			Segments			Lymphocytes		
Hour	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5
Arithmetic mean	0.40	0.38	0.38	7.36	6.75	6.86	10.86	7.53	7.05	0.01	0.00	0.01	0.18	0.19	0.37	0.82	0.82	0.62
Standard deviation	0.04	0.04	0.02	1.56	1.09	0.79	3.36	2.63	2.43	0.01	0.01	0.01	0.20	0.15	0.10	0.19	0.15	0.13
Pair *t*-test (*P*)	0.158[a]	0.115[b]	–	0.276[a]	0.466[b]	–	0.024*[a]	0.023*[b]	–	0.559[a]	0.225[b]	–	0.935[a]	0.024*[b]	–	0.829[a]	0.017*[b]	–

*$P < 0.05$; [a]*P*-value of pair *t*-test of values at 0 vs. 1.5 h; [b]*P*-value of pair *t*-test of values at 0 vs. 5 h

Table 7. Control group – blood gases

	pH			p_aCO_2 (kPa)			p_aO_2 (kPa)			HCO_3^- (mmol/l)			Base excess (mmol/l)			S_aO_2 (%)			$p_{A\text{-}a}O_2$ (kPa)		
Hour	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5
Arithmetic mean	7.42	7.50	7.45	5.56	4.55	4.69	9.12	10.98	12.39	26.8	26.5	24.2	2.7	2.2	–0.5	94.8	99.3	99.3	3.81	3.39	2.63
Standard deviation	0.03	0.05	0.06	0.85	0.99	0.99	0.60	1.90	1.82	2.4	2.2	2.6	2.5	2.4	2.9	0.9	0.9	1.0	0.38	0.77	0.19
Pair *t*-test (*P*))	0.006*[a]	0.208[b]	–	0.010*[a]	0.050[b]	–	0.009*[a]	0.001*[b]	–	0.263[a]	< 0.001*[b]	–	0.136[a]	< 0.001*[b]	–	0.001*[a]	< 0.001*[b]	–	0.150[a]	< 0.001*[b]	–

*$P < 0.05$; [a]*P*-value of pair *t*-test of values at 0 vs. 1.5 h; [b]*P*-value of pair *t*-test of values at 0 vs. 5 h

Table 8. Control group – ions

	Na^+ (mmol/l)			K^+ (mmol/l)			Ionized Ca^{2+} (mmol/l)			Cl^- (mmol/l)			AnGap		
Hour	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5	0	1.5	5
Arithmetic mean	137.3	137.0	136.9	4.38	4.83	4.09	1.29	1.42	1.74	103.0	105.6	106.8	10.3	9.4	9.4
Standard deviation	1.2	2.2	2.2	0.81	0.45	0.40	0.05	0.04	1.19	2.5	2.2	2.2	2.5	4.1	2.3
Pair *t*-test (*P*)	0.703[a]	0.628[b]	–	0.022*[a]	0.129[b]	–	0.001*[a]	0.282[b]	–	0.002*[a]	< 0.001*[b]	–	0.394[a]	0.256[b]	–

*$P < 0.05$; [a]*P*-value of pair *t*-test of values at 0 vs. 1.5 h; [b]*P*-value of pair *t*-test of values at 0 vs. 5 h

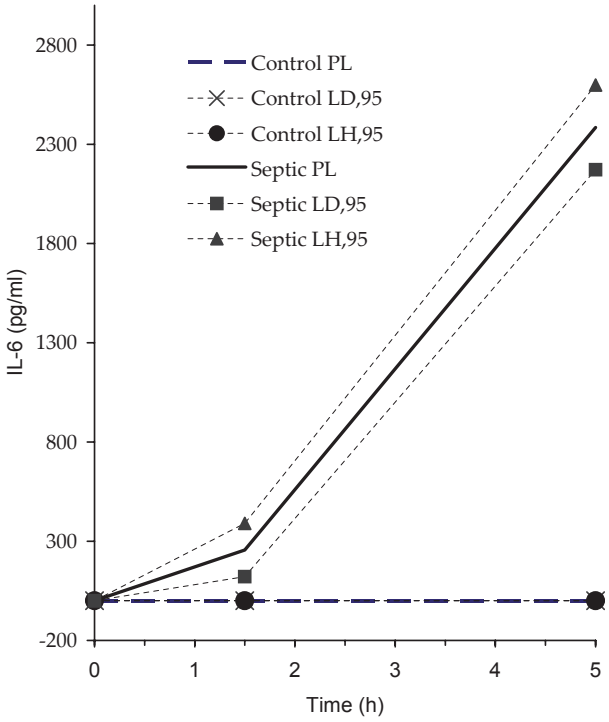

Figure 1. Distribution of IL-6 levels – septic shock vs. control group

LD,95 = lower pivot in 95 percentile; LH,95 = higher pivot in 95 percentile; PL = semisum of LD,95 and LH,95

DISCUSSION

The diagnosis of systemic inflammatory response syndrome (SIRS) in clinical practice is frequently based on clinical signs and routine laboratory examinations. Common markers of inflammation are body temperature, heart and respiratory rates and the white blood cell count. The syndrome of systemic inflammatory response, nevertheless, may be of both infectious and non-infectious aetiology (Bone et al., 1992). The alteration of cardinal signs, despite their sensitivity, is a rather non-specific criterion due to variable aetiology. The low specificity of the white blood cell count is documented by the criterion of presence of either leukopenia or leukocytosis, and left shift, in the definition of sepsis and SIRS (Bone et al., 1992).

The inconsistent development of the triage (i.e., temperature, respiratory and heart rates) in the monitored groups of experimental animals serves to document variability of these parameters under the influence of many factors. Both groups of animals showed a marked drop in body temperature, probably due to insufficient thermoregulation during the anaesthesia. Pulse rates had a significant tendency towards bradycardia in the septic group compared with the control. The drop in the respiratory rates of septic animals at the end of the study may be explained by the higher mortality in this group. Many individuals in this group displayed pathological breathing such as gasping and apnoic pauses which are common just prior to cardiopulmonary arrest (Muir, 1999; Hendricks, 2003). Although the aim was to minimize external factors influencing triage (no use of heated pads for keeping laboratory rats, no corrections for fluid loss through evaporation and urine), the general anaesthesia by itself and the individual susceptibility of the animals to anaesthesia were important interfering factors (Holden and Hammond, 1999). Therefore, the evaluation of cardinal signs in animals under general anaesthesia in relation to the development and course of shock and SIRS has only a limited importance in examining, in particular, absolute values. Greater benefit may rather be expected from examining the trends of their development, as shown in general rules of monitoring critically ill patients (Kaplan, 1992; Hammond and Walters, 1999).

Decreasing white blood cell counts in the septic shock group may be due to the development of SIRS in response to the endotoxin released from *E. coli* (Bone et al., 1992; Green and Adams, 1992; Haskins, 1992). In the septic shock group there was a rise in bands, significant when compared with the control, as well as changes in the proportion of segmented neutrophils and lymphocytes at the end of the study. Metabolic acidosis was also gradually developing in this group of animals. The functions of the lung parenchyma, measured by the p_aO_2, S_aO_2 and $p_{A\text{-}a}O_2$ parameters, did not change in both groups of animals monitored. Metabolic acidosis accompanies the development of SIRS and the analysis of blood gases and/or parameters of anaerobic metabolism, as confirmed by prior studies, may be suitable not only for the evaluation of the actual state of the patient but also for prognostic purposes (Smith et al., 2001; Marecaux et al., 1996). The increased anion gap values in the SS group at the end of the experiment correspond with the metabolic acidosis and the drop in the concentration of bicarbonates.

Evaluating glycaemia in the septic shock group, it is clear that there were no significant changes leading to a rise or drop in blood glucose except for

some broadening of the range of values measured (increased standard deviation). This fact, however, was not helpful in distinguishing between the septic and normal groups of animals. In the course of shock and SIRS there are changes in the metabolism of lipids and glucose due to the release of glucocorticoids and adjustments in the release and action of insulin. Levels of glucose are then the result of the ratio between the effects of corticosteroids and insulin.

A recent study on the insulin therapy of children with burns has demonstrated the anti-inflammatory effects of insulin and its positive effect on a course of therapy (Jeschke et al., 2004). From previous studies on sepsis it is known that bacterial endotoxins interfere significantly with the action of insulin at the cellular level and may cause hyperglycaemia as well as hypoglycaemia in septic patients (Das, 2003; Yu et al., 2003).

As expected, a significant rise in the IL-6 level was found in the septic shock group. The rise of serum IL-6 levels during sepsis and septic shock states has been reported in many human clinical studies as well as experimental studies employing animal models. Hack et al. (1989) described increased levels of IL-6 in septic patients and significantly higher levels in patients in septic shock. In these patients IL-6 levels had a prognostic value because they were significantly higher upon admission in patients who died than in those who survived. However, levels of IL-6 were not influenced by the source of infection (Hack et al., 1989). Similarly, in an experimental study employing dogs the level of cytokines was not influenced by the site of infection (Moeniralam et al., 1997). Calandra et al. (1991) also reported increased levels of IL-6 in septic patients and significantly higher values in patients who were dying and showing signs of a fulminant septic shock than in patients who expired after a temporary clinical improvement or patients who recovered. IL-6 levels were at their maximum upon admission, decreased during the stay in the hospital and correlated with the length of hospitalization. Nevertheless, it was not possible to determine values limiting the survival of individual patients. Another study which reported higher IL-6 levels in septic compared to non-septic shock patients found that survival was not correlated with levels of IL-6 but with the duration of the increased levels (Pinsky et al., 1993). Martin et al. (1997) also reported IL-6 levels higher in septic than in non-septic (traumatic) shock patients. At the same time they found rising levels of IL-6 in a polytraumatic patient who developed pneumonia during hospitalization. Recently, Martins et al. (2003) confirmed the prognostic importance of IL-6 during the late stages of sepsis and septic shock. All the above studies are based on the results of IL-6 levels measured upon the arrival of patients at the hospital and then at regular intervals throughout their hospitalization; the highest levels are mostly those obtained on arrival.

Levels of IL-6 in dogs following the administration of *E. coli* endotoxin rose significantly after 90 min and peaked within 180 min without regard to the site of injection or the general anaesthesia (Moeniralam et al., 1997). Similar results were obtained by Forfia et al. (1998) who reported a significant rise in IL-6 levels in dogs 120–240 min after bacterial lipopolysacharide (LPS) administration, while the IL-6 levels also peaked within this time range (Forfia et al., 1998). A study was also conducted on the effect of hypothermia on inflammatory responses in laboratory rats. A significant rise in IL-6 levels was observed some 120 min after the administration of LPS from *E. coli* in a dose which induced a shock which increased slightly after 300 min. IL-6 levels diminished with the growing degree of hypothermia (Taniguchi et al., 2003). Contrary to human clinical studies, experimental work using animals as a model of the development of sepsis report a peak of IL-6 which climbs from basal levels following the induction of septic states (administration of LPS).

The results of this study on the dynamics of septic shock are in concord with previous papers monitoring the development of sepsis and septic shock. Considering the relationship between IL-6 and sepsis, it can be stated that there is a clear response to the insult during the early stages of shock, while the rise of this parameter starts just prior to changes in other laboratory parameters such as glycaemia, metabolic acidosis, or blood differential count – a sensitive marker of inflammatory responses during sepsis. The use of live *E. coli* to induce sepsis means that this model is more representative of the real conditions during sepsis and septic shock under clinical practice.

The experimental part of the study did have certain limitations, such as the number of animals used in the septic shock group. This may, in particular, have influenced results and explained how some parameters did not change profoundly during shock. The prolongation of the experimental work

would also be helpful in shedding more light on lower responding parameters during shock. Higher numbers of experimental animals would also enable the evaluation of selected parameters in relation to the prognosis of the disease. The number of animals in this study was limited by the relative cost of determining serum IL-6 levels. Clinical parameters may also be considerably influenced by procedures performed during the experiment such as the interference of general anaesthesia with cardinal signs which are consequently of low informative value.

CONCLUSION

The model of septic shock induction in the laboratory rat using systemic distribution of live *E. coli* (O18) presented in this paper proved to be functional and confirmed the importance of IL-6 in the pathogenesis of septic shock.

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