ONCOLOGY

Dynamics of Elimination of Bacterial Endotoxins and Cytokines from the Blood of Tumor Patients with Sepsis in Hemoperfusion using Carbon Adsorbents

N. Yu. Anisimova, E. G. Gromova, L. S. Kuznetsova, S. M. Sitdikova, and M. V. Kiselevskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 151, No. 5, pp. 560-562, May, 2011
Original article submitted February 9, 2010

We studied the effects of carbon adsorbent hemoperfusion on the dynamics of LPS and cytokine concentrations in the blood of cancer patients with sepsis and septic shock. In addition, hemadsorbent washout fluid specimens after hemoperfusion were analyzed. A significant (3-fold) reduction of blood LPS levels after hemoperfusion was found, while shifts in free cytokine levels in the sera were negligible. At the same time, hemadsorbents used in the study effectively eliminated some free cytokines (IL-6, -8, -12, IFN-γ, TNF-α) from the blood.

Key Words: *hemoperfusion; adsorbent; lipopolysaccharide; cytokines; sepsis*

Sepsis responsible for high mortality of patients with immunodeficiency requires therapeutic procedures providing rapid and effective elimination of inflammation factors and mediators. LPS from gram-negative bacterial wall and some proinflammatory cytokines, including those indirectly stimulated by LPS, are important mediators of sepsis, triggering the cascade of homeostasis disorders. Hemoperfusion (HP) was suggested as a medical procedure in the 1960s [4]. One of the materials first suggested as an adsorbent was activated carbon characterized by large effective area (500-1500 m^2/g) due to its porous surface and chemical inertness. Due to progress in the development of new and modification of previously suggested adsorbents, hemoperfusion as a method for blood cleansing has many advantages over other nonselective methods of extracorporeal detoxication (plasmapheresis, plasma filtration) [4]. Development of a technology for obtaining activated carbon by pyrolysis of synthetic polymers, which improved significantly the biocompatibility of this substance and its stability in biological fluids, was one of the facts largely determining these advantages. HP columns manufactured at present contain granulated activated carbon coated with cellulose (Norit RBX B Adsorba 300C and Adsorba 150C, Gambro), poly-HEMA (Hemosorba, Asahi Medical and Nextron Medical Technologies) or hydrogel with heparin (Clark R&D) [2,3,5].

We evaluated the effects of HP on the dynamics of blood concentrations of LPS and a wide spectrum of cytokines in cancer patients with sepsis and septic shock.

MATERIALS AND METHODS

The study was carried out on blood specimens from 14 cancer patients with sepsis and septic shock treated by extracorporeal detoxication by means of HP with Adsorba 300 hemadsorbents (Gambro) at Intensive Care Department No. 2 of N. N. Blokhin Oncological

N. N. Blokhin Russian Oncological Research Center, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** n.u.anisimova@gmail.com. N. Yu. Anisimova

0007-4888/11/1515-0622

Research Center. The adsorbent washout fluid specimens after the procedure were also analyzed.

The blood was collected before and after HP. The serum was separated and stored at -70°C until analysis. Adsorbent washout fluid after HP procedure was collected as follows. Adsorbent specimen (2 g) was removed from the column, plunged in saline, and shaken on an orbital shaker (350 rpm) for 30 min. After centrifugation, the supernatant was collected and stored at -70°C. The concentration of LPS was measured by LAL test with commercial kits (Hycult Biotechnology). Cytokines were measured by ELISA kit (Bender MedSystems).

Statistical analysis was carried out by Statistica 6.0 software (StatSoft).

RESULTS

Bacterial endotoxin (LPS), a component of gram-negative bacterial cells and product of their degradation, leads to hyperproduction of inflammation mediators, including cytokines, and plays an important role in the pathogenesis of sepsis. Circulation of excessive endogenous mediators induces disorders leading to the development of organ and polyorgan failure.

In 9 patients, HP procedure reduced LPS concentrations in the blood. In 3 patients with initially low LPS concentrations (0-0.08 U/ml) this parameter remained unchanged. In 2 patients LPS concentrations increased directly after the end of the procedure. Figure 1 presents the effects of HP on serum LPS levels in each patient participating in the study.

The results of comparative analysis of the dynamics of LPS concentrations in the blood of patients during extracorporeal detoxication and in the adsorbent washout fluid are presented in Figure 2. The data indicate effective elimination of LPS from patient blood with Adsorba 300 adsorbent (3-fold reduction of LPS concentration). High levels of LPS were found in the adsorbent washout fluid specimens.

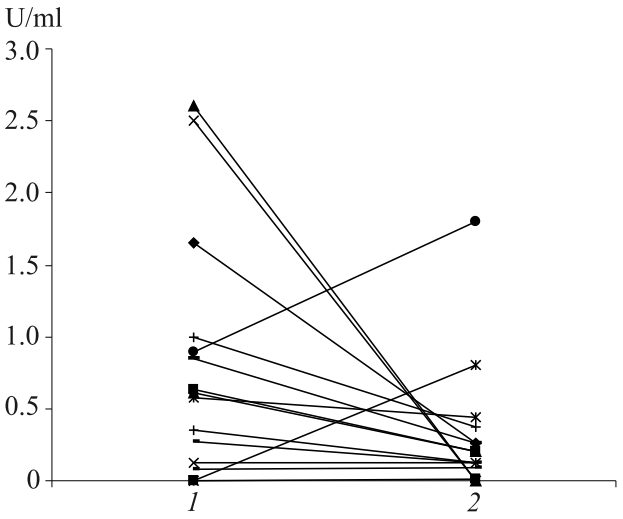

Fig. 1. Dynamics of blood LPS concentrations in cancer patients with sepsis and septic shock before (*1*) and after (*2*) HP.

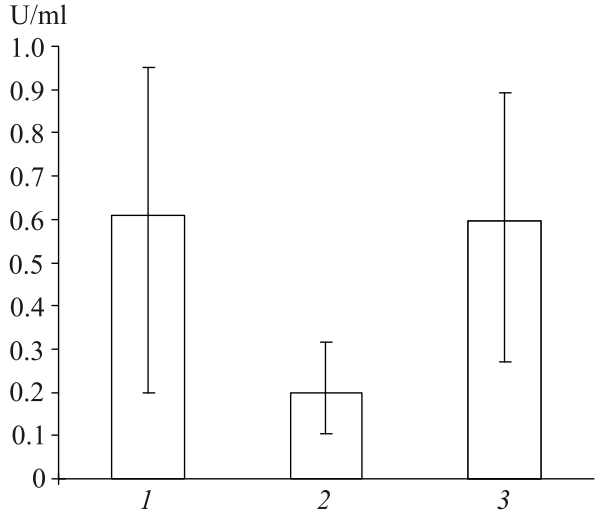

Fig. 2. Dynamics of blood LPS concentrations in cancer patients with sepsis and septic shock before (*1*) and after (*2*) HP and in adsorbent washout fluid (*3*).

TABLE 1. Blood Concentrations of Pro- and Anti-Inflammatory Cytokines in Cancer Patients with Sepsis and Septic Shock before and after HP

Time of blood collection	IL, pg/ml				IFN-γ, pg/ml	IL-12, pg/ml	TNF, pg/ml		IL, pg/ml		
	6	8	10	17			β	α	1	4	5
Before HP	59 (57-747)	6 (4-23)	41 (28-56)	91 (47-194)	21 (19-24)	62 (50-65)	22 (0-72)	60 (38-222)	0 (0-54)	29 (27-36)	77 (61-88)
After HP	65 (46-704)	9 (4-14.25)	51 (36-81)	120 (57-178)	19 (15-20)	56 (18-81)	2 (0-65)	68 (25-127)	0 (0-43)	30 (26-39)	79 (63-82)

Note. The data are presented as the median and 25 and 75 quartiles.

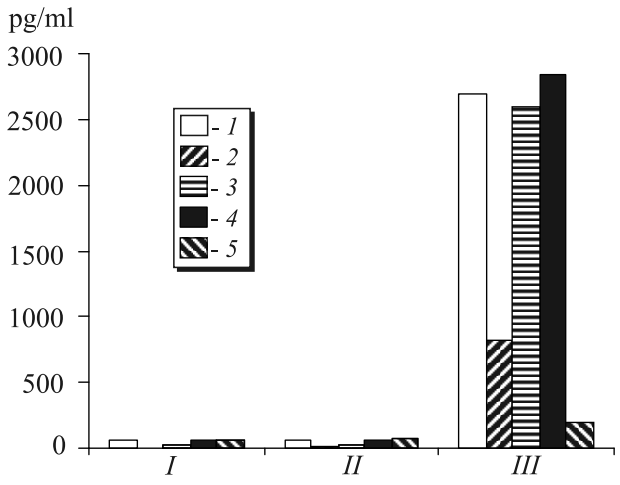

Fig. 3. Dynamics of cytokine concentrations in the blood of cancer patients with sepsis and septic shock before (*I*) and after (*II*) HP and in adsorbent washout fluid (*III*). *1*) IL-6; *2*) IL-8; *3*) IFN-γ; *4*) IL-12; *5*) TNF-α.

No appreciable changes in serum levels of free cytokines after HP were found (Table 1). However, the absence of changes in serum cytokine concentrations after HP does not indicate that carbon adsorbents poorly reduce the cytokine concentrations in biological liquids, as high levels of IL-6, -8, -12, IFN-γ, and TNF-α were found in the adsorbent washout fluid after HP (Fig. 3).

A trend to a considerable (3-fold on average) reduction of blood LPS concentrations as a result of HP was observed. However, in 2 patients the concentration of bacterial endotoxin increased after the procedure. This could be caused by enhanced translocation of enteric microflora endotoxins from the intestine and by destruction of LPS-protein complex during HP, which masked high endotoxin level in the blood. In addition, those two patients presented with septic shock (in contrast to other patients) before HP. One female patient died within 24 h after the procedure, the other patient was subjected to repeated HP, which led to improvement of his clinical status and laboratory values, including reduction of blood LPS concentration. Improvement of the clinical status was observed in the rest 12 patients participating in the study. Presumably, this positive clinical effect was also due to elimination of excessive cytokines from the blood. Paradoxical absence of changes in the levels of free serum cytokines and high levels of IL-6, -8, -12, IFN-γ, and TNF-α in the adsorbent washout fluid could be due to cleavage of the cytokine-protein complex and subsequent effective adsorption of free cytokines. Some authors had hypothesized "latent cytokinemia" in septic patients, circulation of bound cytokine forms in the blood, which could not be identified by commonly used EIA commercial kits [1].

Hence, the data indicate the efficiency of carbon hemadsorbents and provide pathogenetic validation for their use for extracorporeal therapy of cancer patients with sepsis.

REFERENCES

1. A. R. Tuguz, E. G. Gromova, N. V. Malakhova, *et al.*, *Immunologiya*, No. 5, 56-58 (2001).
2. C. A. Howell, S. R. Sandeman, and G. J. Phillips, *Biomaterials*, **27**, No. 30, 5286-5291 (2006).
3. M. Melillo, G. J. Phillips, J. G. Davies, *et al.*, *Carbon*, **42**, No. 3, 565-571 (2004).
4. S. V. Mikhalovsky, *Perfusion*, **18**, No. 1, 47-54 (2003).
5. T. Tabei, Y. Akai, S. Takeda, *et al.*, *Biomater. Artif. Cells Immobilolization Biotechnol.*, **19**, No. 1, 193-201 (1991).