METHODS

Possibility of Microorganism Elimination from the Blood Using Modified Coal Hemosorbents

N. Yu. Anisimova, T. S. Spirina, K. S. Titov, N. V. Malakhova, S. M. Sitdikova, and M. V. Kiselevsky

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We studied the possibility of using coal hemosorbents for elimination gram-positive and gram-negative microorganisms from the blood. After hemosorption, the number of *S. aureus* and *K. pneumoniae pneumoniae* colony-forming units significantly decreased. The obtained results indicate that coal sorbents can bind and probably eliminate gram-positive and gram-negative bacteria from the blood.

Key Words: *sorbent; bacteria; sepsis; hemosorption*

The possibility of eliminating LPS (cell wall component of gram-negative bacteria) from biological fluids using various modifications of coal sorbents was previously reported [1-3]. However, we found no data on the possibility of eliminating bacterial cells from the blood during hemosorption (HS). This problem is of particular importance, because bacteriemia is verified in a substantial part of patients with diagnosed sepsis.

The aim of this study was to evaluate the possibility of eliminating various microbial cells from body fluids using coal sorbents.

MATERIALS AND METHODS

Suspensions of 20-h bacterial cultures diluted to 0.5 U according to McFarland turbidity scale were used in the experiments. The suspension of gram-positive (*S. aureus*) and gram-negative (*K. pneumoniae pneumoniae*) microorganisms in a volume of 5 ml was added to the test tubes with blood from healthy donors (450 ml) and the mixture was passed under pressure through a model of hemosorption column filled with coal sorbent (10 g), an active base material of Adsorba 300 (Gambro). Then equal volumes of blood after HS and blood which did not contact with the sorbent (control) were inoculated on dishes with solid medium. All procedures were conducted under sterile conditions. The results were analyzed after 24 h. Index of inhibition of colony formation (ICF) for the hemoculture following HS was calculated according to the formula:

$$ICF=CC_{after\ HS}/CC_{control}\times 100\%,$$

where CC is mean number of colonies per 1 cm^2 culture surface (at least 10 squares were counted). The study presents pooled data obtained in three experimental series. Each experiment was conducted using the data obtained after inoculation of hemoculture from one of three donors. The data are presented as mean±standard deviation.

RESULTS

In control series, the bacterial culture actively utilized mannite from the medium. This led to acidification of

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

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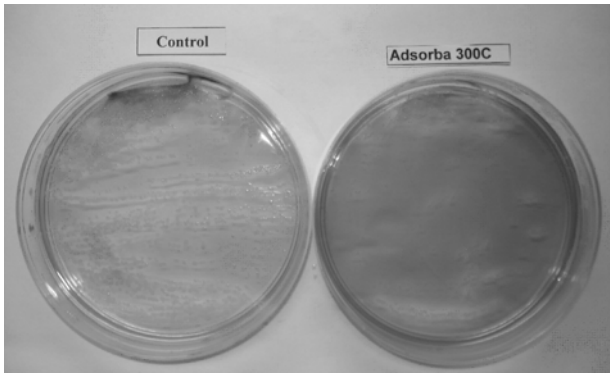

Fig. 1. Intensity of colony formation in *S. aureus* culture before (control series) and after HS on mannite-salt agar.

the medium and to corresponding change of agar color from pink to yellow. The blood after HS yielded less intensive growth of *S. aureus* colonies, which manifested in insufficient change of agar color (Fig. 1).

After HS, a sufficient decrease of *S. aureus* and *K. pneumonia pneumonia* colony formation was observed (ICF 65% and 68%, respectively), which attested to a considerable decrease in the content of microbial CFU in the blood passed through the tested coal sorbent.

The study revealed reduced content of *S. aureus* and *K. pneumoniae pneumoniae* CFU after HS. These microorganisms have different size, shape, and structural and biochemical properties. It seems likely that the observed decrease in colony formation is a result of binding of bacterial cells to the sorbent. Bearing in mind that modified activated coal sorbents bind LPS, a structural unit of cell wall in gram-negative bacteria, elimination of K. *pneumoniae* from the body fluid seems to have a logical basis, although the mechanism of this effect remains disputable, because modern hemosorbents, along with high indices of biocompatibility, possess low sorption properties for molecules with high (>15,000 Da) and medium molecular weight (300-15,000 Da) [6].

The possibility of elimination of gram-positive bacteria from the blood is also a very intriguing fact. Hence, the positive effects of HS for the treatment of patients with bacteriemia caused by both gram-positive and gram-negative bacteria can be anticipated. Effective removal of proinflammatory cytokines from biological fluids by coal sorbents was previously reported [4,5,7]. Together with the known capacity of hemosorbents to eliminate inflammation-initiating factors (bacteria and their toxins) from the blood, this fact allows regarding the use of HS in the treatment of patients with sepsis as highly promising.

REFERENCES

1. M. Melillo, G. J. Phillips, J. G. Davies, *et al., Carbon*, **42**, No. 3, 565-571 (2004).
2. S. V. Mikhalovsky, *Perfusion,* **18**, Suppl. 1, 47-53 (2003).
3. M. C. Murphy, S. Patel, G. J. Phillips, *et al*., *Studies in Surface Science and Catalysis, Characterisation of Porous Solids* V.I., Eds F. Rdriguez-Reinoso *et al*., Amsterdam, 515-520 (2002).
4. C. Ronco, A. Brendolan, M. Dan, *et al., Kidney Int.,* **58**, S148-S155 (2000).
5. S. R. Sandeman, C. A. Howell, S. V. Mikhalovsky, *et al., Biomaterials*, **29**, No. 11, 1638-1644 (2008).
6. R. Vanholder, R. De Smet, P. Vogeleere, and S. Ringoir, *Artif. Organs*, **19**, No. 11, 1120-1125 (1995).
7. G. Yushin, E. N. Hoffman, M. W. Barsoum, *et al., Biomaterials*, **27**, No. 34, 5755-5762 (2006).