In Vitro Effects of Sporobacterin Probiotic on the Function of Donor Granulocyte-Macrophage Cells

N. I. Gabrielyan, V. S. Suskova, S. I. Suskov, and N. L. Vologodskaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 653-655, May, 2012
Original article submitted March 1, 2011

The effects of sporobacterin probiotic (Bakoren Company) on oxidative activity of donor granulocyte-macrophage cells (GMC) were studied *in vitro* by luminol-dependent chemiluminescent method, and the effects of the probiotic on the production of pro- and anti-inflammatory cytokines were evaluated by ELISA. The probiotic dose-dependently stimulated spontaneous production of free radicals by GMC; combined treatment with immunomodulators likopid, polyoxydonium, and IFN-α2a produced a more potent effect. Sporobacterin stimulated the production of pro- and anti-inflammatory cytokines in cell cultures. These data confirmed the immunomodulatory effect of sporobacterin, an important component in the phagocytic system cells.

Key Words: *probiotics; sporobacterin; chemiluminescence; granulocyte-macrophage cells*

High level of generalized infectious complications and mortality in surgical treatment, particularly in cardiosurgery, and the difficulties of the agent eradication, despite the use of the latest generation antibiotics, necessitate the search for new trends and methodological approaches to the problem of effective prevention and therapy of infectious postsurgical complications [4,8]. One of these trends is use of probiotics, biological preparations created from living apathogenic microorganisms, representatives of the normal microflora or environment, exhibiting positive effects on the physiological, biochemical, and immune reactions of the host [1,4,7].

Sporobacterin probiotic based on *B. subtilis* 534 has been developed in Russia and since 1992 is allowed for clinical use in the treatment of dysbiotic conditions and pyoinflammatory processes. The experience gained in the use of sporobacterin in surgical practice indicates a significant reduction of the incidence of postoperative pyoinflammatory complications, including those in cardiosurgical patients [2].

V. I. Shumakov Center of Transplantology and Artificial Organs, Moscow, Russia. ***Address for correspondence:*** sis_tio@mail.ru. V. S. Suskova

On the other hand, the immunomodulatory effect of sporobacterin remains little studied, which prompted us to study its effects on the function of the phagocytic system, playing the key role in the development of pyoinflammatory complications.

MATERIALS AND METHODS

The effects of sporobacterin probiotic on the function of the phagocytic system were studied *in vitro* on leukocyte suspension of heparin-treated blood from 25 blood donors (13 men and 12 women aged 37 (28-46) years without disorders in the neutrophilic phagocytic function). Cell viability was evaluated by the Trypan Blue test and was 95-98%.

Oxidative activity of granulocyte-macrophage cells (GMC) was evaluated by luminol-dependent chemiluminescence (CL) on a Lucy2 chemiluminometer using PC software. The dose-dependent effects of sporobacterin (Bacoren, series No. 160309; initial concentration 10^9 spores/ml) on the activity of myeloperoxidase system, reflecting the GMC bactericidal activity, were studied by adding 10 and 20 μl probiotic to intact leukocyte suspension (10^6 cell/ml, microplate variant) as the stimulant, after which

0007-4888/12/1535-0697

the samples were cultured for 30 min at 37°C and 5% CO_2.

Sporobacterin effect was compared to the intensity of spontaneous CL of neutrophils/monocytes without stimulant.

Luminol (5-amino-2,3-dihydro-1,4-phthalasine; Sigma) in the final concentration of 24.5 μM (V=100 μl) served as the fluorescence stimulant. The CL intensity (mV) was evaluated by the maximum amplitude of the signal over t=10 sec (1 signal per sec) from the moment (t=0) of the preparation addition and was expressed as the stimulation index (SI)=CL_{exp}/CL_{contr}, where CL_{exp} was CL intensity in the sample with the preparation and CL_{contr} CL intensity in the control.

Sporobacterin effect on GMC oxidative activity was compared with the effects of common immunomodulators (likopid, cycloferon, polyoxydonium, imunofan, IFN-α2a) in therapeutic doses. The effects of sporobacterin in combination with polyoxydonium (polyethylene piperazine N-oxidized derivative; Immunopharma), likopid (Peptek), and IFN-α2a (Vector) were studied.

Sporobacterin effect on spontaneous and LPS-induced production of IL-1β, TNF-α, IL-6, IL-10, IL-1Ra was studied on heparin-treated (20 U/ml) whole blood (1:5 dilution) cultured in complete RPMI-1640 with 1 μg 0.005% pyrogenal or 20 μl sporobacterin per 100 μl of a test sample. Pyrogenal (LPS isolated from *Salmonella typhi*; endotoxin stimulating GMC activity; Medgamal Affiliated Dept., N. F. Gamaleya Institute of Epidemiology and Microbiology) was used to stimulate the cytokine production. Culturing was carried out for 18 h at 37°C and 5% CO_2. The measurements were carried out by EIA (Cytokine) on a Picon spectrophotometer [5].

The protocol of the study was approved by the Ethic Committee of V. I. Shumakov Center of Transplantology.

The results were statistically processed using applied software. The significance of differences was evaluated by Student's *t* test. The differences were considered significant at $p<0.05$.

RESULTS

Evaluation of oxidase activity of donor blood GMC under the effects of various sporobacterin doses *in vitro* showed a significant dose-dependent increase in functional activity of these cells. The CL intensity increased 2- and 4-fold, respectively, in comparison with the control in response to 10 and 20 μl sporobacterin added to the leukocyte suspension and by 2.5 times in comparison with the higher concentration of the probiotic (Fig. 1).

The fluorescence intensity amplitude in response to 20 μl sporobacterin was constant throughout the entire period of signal recording, which suggested considering it optimal and use in subsequent studies.

Comparison of sporobacterin oxidative activity with that of widely used immunomodulators showed that sporobacterin stimulation index (SI=1.6) did not differ from cycloferon index (1.5) and was lower than polyoxydonium (2.8) and imunofan (1.9) indexes, being intermediate; this confirmed its immunomodulatory effect.

Studies of sporobacterin efficiency in combinations with immunomodulators showed a significant ($p<0.05$) increase of the intensity of luminol-dependent CL reflecting activity of GMC myeloperoxidase system (Fig. 2) only for sporobacterin combinations

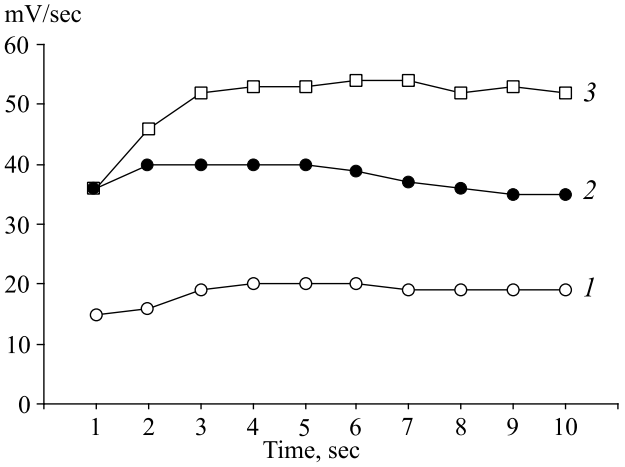

Fig. 1. Intensity of luminol-dependent CL of donor GMC in response to different sporobacterin doses. *1*) control (no sporobacterin); *2*) sporobacterin, 10 μl; *3*) 20 μl. $p<0.05$ between *1* and *2*, *1* and *3*, *2* and *3*.

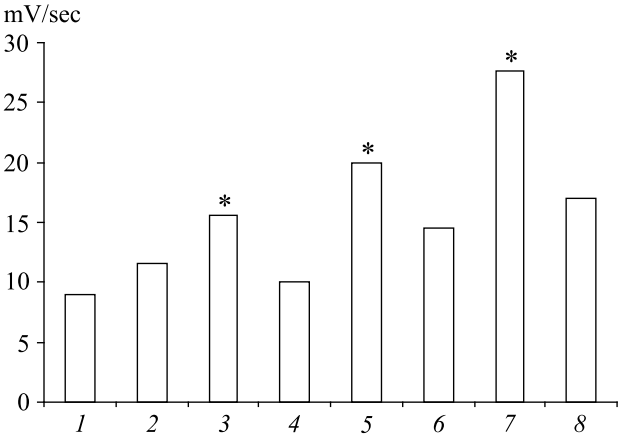

Fig. 2. Intensity of luminol-dependent CL of GMC under conditions of induction by sporobacterin and its combinations with immunomodulators. All immunomodulators in a dose of 20 μM. *1*) spontaneous CL; *2*) sporobacterin; *3*) sporobacterin+likopid; *4*) likopid; *5*) sporobacterin+IFN-α2a; *6*) IFN-α2a; *7*) sporobacterin+polyoxydonium; *8*) polyoxydonium. *$p<0.05$ in comparison with *2*.

TABLE 1. Effects of Sporobacterin on Production of Pro- and Anti-Inflammatory Cytokines *In Vitro* (Me [min-max])

Cytokines	Spontaneous	Spontaneous+sporobacterin	LPS-induced
Proinflammatory			
IL-1β	32 (30-34)	71* (44-96)	147^{+} (80-214)
IL-6	239 (73-437)	432* (349-476)	477* (379-479)
TNF-α	136 (103-178)	352* (267-460)	416* (250-729)
Anti-inflammatory			
IL-10	176 (78-275)	325* (156-656)	258* (100-492)
IL-1Ra	252 (155-451)	455* (189-647)	413* (326-475)

Note. $p<0.05$ in comparison with: *spontaneous culture, $^{+}$sporobacterin stimulation.

with likopid, IFN-α2a, and polyoxydonium, modulators stimulating the function of the phagocytic system [5,7].

The results of comparative analysis of the production of pro- (IL-1β, IL-6, TNF-α) and anti-inflammatory cytokines (IL-10, IL-1Ra) in spontaneous and sporobacterin- and LPS-induced cultures of donor peripheral blood are presented in Table 1.

The *in vitro* production of all the studied cytokines in response to sporobacterin stimulation 2-2.5-fold surpassed the initial level ($p<0.05$) and was comparable with the responce to LPS stimulation. This confirmed the cytokine-mediated mechanism of the probiotic effect through GMC stimulation providing the antibacterial and anti-inflammatory response.

These data were in good agreement with sporobacterin effect on the GMC oxidative activity in luminol-dependent chemiluminescent test reflecting the bactericidal effects of sporobacterin, its synergic activity with immunomodulators (IFN-α2a, likopid, and polyoxydonium), whose action mechanisms are based on affinity for bacterial membrane structures [3,6,8].

Hence, the results have confirmed the immunomodulatory effects of sporobacterin as a preparation improving the function of the phagocytic system cells.

REFERENCES

1. V. M. Bondarenko, R. P. Chuprynina, and M. A. Vorobyova, *Biopreparaty*, No. 3, 2-5 (2003).
2. V. I. Shumakov, N. I. Gabrielyan, E. A. Gorskaya, *et al.*, *Invention Patent No. 2350329. A Method for Prevention of Postoperative Pyoseptic Complications in Cardiosurgery.*, *Byull. Otkrytiya i Izobreteniya*, No. 9, 27.03.2009.
3. E. V. Men'shikova, N. K. Zenkov, V. Z. Lankin, *et al.*, *Oxidative Stress. Pathological Conditions and Diseases* [in Russian], Novosibirsk (2008).
4. G. G. Onishchenko, V. A. Alyoshkin, and S. S. Afanasyev, *Immunobiological Preparations and Prospects for Their Use in Infectology* [in Russian], Moscow (2002).
5. R. M. Khaitov and B. V. Pinegin, *Immunologiya*, No. 5, 4-7 (2000).
6. S. Briganti and M. Picardo, *J. Eur. Acad. Dermatol. Venerol.*, **17**, No. 6, 663-669 (2003).
7. W. H. Holzapfel, P. Heberer, R. Geisen, *et al.*, *Am. J. Clin. Nutr.*, **73**, No. 2, Suppl., 365S-373S (2001).
8. P. Rocha-Pereira, A. Santos-Silva, I. Rebelo, *et al.*, *Clin. Chim. Acta*, **303**, Nos. 1-2, 33-39 (2001).