LITERARY SOURCES CITING EAR, NOSE AND THROAT STUDIES USING PROBIOTICS
BY L. SHYNKARENKO-SICHEL AND D. ZABOLOTNY

1. **Probiotic modulation of the immunogenesis in the experiment; Zabolotny D., Melnilkov O., Zabolotnaya D., Shinkarenko L.** ***Immunology and allergology №1, 2006 ., p.19-21***

Materials and methods

Probiotic strain drugs Lactobacillus murinus and Lactobacillus rhamnosus. The strains are distinguished and selected at the department of the industrial biotechnology of the STUU (Scientific Technological University of Ukraine) KPI (Kiev, Ukraine), they are deposited in the repository of the Institute of Microbiology and virology of NAS (national Academy of Science) of Ukraine. The medications of ionophilic-dried cells of lactic acid bacilli of Lactobacillus murinus (LE) strains were under investigation in the concentration of 5 10 9 cell\dose and Lactobacillus rhamnosus (LB3) with the same concentration, as well as complex medication containing the same strains in the above mentioned doses (LE+ LB3) was studied.

New forms of the lactic acid bacilli drugs demonstrate antagonistic activity towards the most frequent microbes found during recrudescence of inveterate lemic-inflammatory diseases of ENT organs, have high degree of conglutination to mucous coat of the upper air passages, are acid and alkaline tolerant, as well as they can produce antimicrobial and antibiotic-like substances.

During 10 days the animals orally took 109 medications.

In the course of the study the potency of the antibody response to the red blood cells of the sheep or chickens was being determined.

Cytolytic activity of the blood cells as to the red blood cells of the chickens was being defined.

The degree of the proportion of the Immunoglobulin Fc fragment receptor cells was also assessed.

The effect of the medications under the conditions of the immunodeficiency was studied. The immunodeficiency was designed using cyclophosphan maneuver in the dose of 50 mg/kg a day before the intake of the probiotic.

Comparator agents–Linex (LEK, Slovenia) and the therapeutic yoghurt (Institute Resell, Canada).

Results

LB3 was most active and on the average stimulated antibody responses three times as much as the controls (minimum–maximum -2,9 – 4,3 times).

Only LB3 demonstrated capacity for the increase of the plasma cells in the blood of the immunodeficient animals - 2,5 times more than the controls (minimum–maximum 2,0-4,6 times).

LB3 also stimulated formation of the FcR+ cells in the blood of healthy animals.

LE stimulated immune cytolysis more intensively in comparison with other medications, it increased cytolytic activity 1,85 times in the healthy animals (minimum–maximum varied within 1,2 - 2, 0) and 2,53 times in the immunodeficient animals (2,1 -2,38).

LE tended to reduce the FcR+ cells in the blood of healthy animals.

Conclusions

Therefore, medications LB3 and LE proved to be more efficient and active compared to the comparator agents. LB3 stimulates humoral immunity responses better.

LE mainly stimulates cell-type responses.

2**. In vitro experimental study of the probiotic effect on the tonsillar cells in the patients with adenoid disease; O.Melnikov,D. Zabolotnaya*... The journal of ear, nose and throat diseases, ,3, 2006, p.24-27.***

Materials and methods

The probiotic medications of Lactobacillus murinus (LE) strains were under investigation in the concentration of 5 10 9 cell\dose and Lactobacillus rhamnosus (LB3) with the same concentration, as well as complex medication containing the same strains in the above mentioned doses (LE+ LB3) was studied. The strains are distinguished and selected at the department of the industrial biotechnology of the STUU (Scientific Technological University of Ukraine) KPI (Kiev, Ukraine), they are deposited in the repository of the Institute of Microbiology and virology of NAS (national Academy of Science) of Ukraine.

The tonsillar cells from the patients with adenoid disease were cultivated with the medications for 4 hours, and then were tested according to the following indexes:

Changes in the number of Immunoglobulin Fc fragment receptor cells;

Changes in the number of cells with membrane antigen CD25 (activated cells) and CD56 (natural cytotoxic cells).

Changes in the number of IgA-producers

Activity level of natural cytotoxic cells

Results

LB3 increased the number of CD25-cells and did not affect the number of CD56-cells.

LB3 increased the number of IgA-producers approximately by 30%.

LB3 intensified the functional activity of the natural cytotoxic cells 2,5-2,7 times as much (maximum -3,75 times).

LE increased the number of CD25-cells (from 2,7 to 2,9) and CD56-cells – on the average –1,45 times(1,7 -1,2).

LE intensified the functional activity of the natural cytotoxic cells 3,4 times as much (from 1,3 to 5 times).

Comparator agent – Canadian yoghurt –did not affect these indexes.

Conclusions

Therefore, medications LB3 and LE proved to be more efficient and active compared to the comparator agents.

LB3 stimulates humoral immunity responses better.

In conditions of the direct contact with effector cells LB3 can increase the activity of the natural cytotoxic cells.

LE mainly stimulates cell-type responses.

Both medications possess activating effect on the wide range of the responders of the tonsillar cells (CD25).

3. **Study of the functional and morphochemical characteristics of cells and tissues under the conditions of the co-cultivation with lactic acid bacilli medication LB3; O.Melnikov, D Zabolotnaya, L. Kalinovskaya, V. Simonenko, 2007, 170-172.....**

National medications conventionally called LB3 and LE, obtained on the basis of Lactobacillus murinus (LE) and Lactobacillus rhamnosus (LB3) strains.
10^5 lactic acid bacilli of LB3 was added to 1 ml of the medium with tonsillar cells. The mixture was cultivated for a day. Then the number of the phagocytes was determined.
It appeared to be 2,9 times more than in the controls and the number of particles absorbed by one cell equaled to 5,8 (average of the phagocyte index) , that is 1,87 times higher than in controls.
The number of cytokines - gamma – interferon was also assessed. of Cytokines - gamma – interferon was synthesized 4,3 higher than in controls.
Interleukine 4 is by 15-20% more than in controls.
IgA - antibody synthesis was intensified 2 times as much with the use of the medication, and the antibody synthesis was intensified в 3,1 times as much.

CONCLUSIONS

The medication can module in vitro phenotypic and functional characteristics of the tonsillar cells:
-activate immunocompetent cells by CD25 antigen,
Increase cellular cytoxity towards heterogenic blasts.
The medication helps to develop valid immune response by 1 type, also inhibits fatty cellular infiltration of the tonsils tissue, reduces the risk of the inflammatory edema.
Stimulates progression of B-cell lymphocytes.
Stimulates progression of high glycogen macrophages
Has inhibitory influence on the nonlymphoid elements, connected with progression of the allergic inflammations.

4. Selection of the excipients for ready-made clinic formulations based on the selected lactic acid bacilli strains; O.Voloska, L. Shinkarenko, T.Todosiichuk, I.Zarytska, D. Zabolotna*, Odessa medical journal. №5 (97), 2006, page.3-6*

Biotherapeutic medications based on the selected strains of Lactobacillus murinus (LE) and Lactobacillus rhamnosus (LB3) were developed for usage in the ENT.
The strains are distinguished and selected at the department of the industrial biotechnology and biological engineering of the STUU (Scientific Technological University of Ukraine) KPI (Kiev, Ukraine), they are deposited in the repository of the Institute of Microbiology and Virology of NAS (National Academy of Science) of Ukraine. - Lactobacillus murinus (LE - №IMB B-7037) and Lactobacillus rhamnosus (LB3 - №IMB –B-7038)

Exsiccation is recommended in producing probiotic preparations LR (local revellents) containing 10%-sucrose and 1%- gelatine. This excipient does not reduce the antagonistic property and survival level of the cultures after cool dehumidification.

5**. Study of the possibility to use lactic acid bacilli in prevention and treatment of the candidiasis of ENT organs; O.Volska, L. Shinkarenko, I.Zarytska, D. Zabolotna; Odessa medical journal,** ***№4 (96), 2006, 32-36***

Materials and methods

In the course of the investigation we used frozen-dried preparations based on lactic acid bacilli containing pure strains of the lactic acid bacteria without culture fluid.
Lactorol - Lactobacillus murinus in the amount of 5 10^9 kl/ml
Acidolor - Lactobacillus rhamnosus in the amount of 5 10^9 kl/ml
Acidolak –mix of the above mentioned cultures in the equal proportions -10^{10} kl/ml
Virtually all the strains of the opportunistic fungi appeared to be sensitive to the medications Candida albicans -90%, Candida krusei – 80%? Candida tropicalis – 100%
Fungicidal activity proved to be the highest in the mixed medication– 1:16,
The fungi neither grew under the direct effect of LE and LB3, cultures nor under the effect of metabolites.
Combined application of the particular antifungal agents with the probiotic medications.

6**. Application of the probiotic medication "Acidolor" in patients with chronic tonsillitis and evaluation of its efficacy; D. Zabolotny, L. Shinkarenko, O.Melnikov, L. Volosevich, I.Zarytska, O.Goloborodko, G. Karpenko, O.Bolyska, D. Zabolotna ,*Journal of ear, nose and throat diseases , №5, 2003, page.2-9***

Medication "Acidolor" obtained on the basis of the lactic acid bacilli Lactobacillus rhamnosus, which are distinguished on the territory of Ukraine is designed at the department of the industrial biotechnology of KPI.

Antagonistic activity towards opportunistic micro flora separated from the patients:

Coccal forms – retardation of growth within 20-30mm

Gram-negative forms– 32-35 mm

Candida fungi -28-30mm

Bactericidal effect varied in concentrations from $0,15\ 10^9$ $1,2\ 10^9$

Lymphoid culture and living culture of the producer possessed similar viscid properties.

Positive result in treatment with the use of the medication was achieved in 88,2% cases, in control group the same parameter equaled 72,8%.

With complex usage of the priobiotec the efficiency increases up to 94,1%

Performance capability increased in all patients (100%), who were prescribed to use mixed priobiotec, and in those who took priobiotec locally, this index is 82,3%.

After the intake of the medication the physiologic flora was restored. The amount of the pathogenic and opportunistic flora was reduced 4-6 times as much.

Bio chemical indexes of the enzymatic activity of the saliva were restored to the natural level, there was observed intensification of the proteolytic activity, the immune parameters were normalized.

7. Perspectives of biotherapy in ENT diseases;D. Zabolotny, L. Volosevich, I.Zarytska, V.D. Zabolotna, *Journal of ear, nose and throat diseases , №3-c, 2003,p.180-181*

For the purposes of the clinic trials the following medications were used: Laktoflor (Ladyzhin plant), Acidolor (KPI»), Biosporin (OJSC »Dniprofarm)
The preparations were prescribed in one dose ($2\ 10^9$ - $5\ 10^9$) once a day during ten days. For ablution of the lacunae of the faucial tonsils in patients with chronic tonsillitis and genyantrums in the patients with maxilloethmoidal sinusitis, the medications were dissolved in 20 ml of normal saline solution.

Results:

Acidolor – positive results in 85,5% cases of the chronic tonsillitis –in maxilloethmoidal sinusitis– 90%
Laktoflor– 81,3 and 80%
Biosporin - 50% and 50%
Controls – antibiotics and antimitotic substances –positive results in 72,8% cases.
Postive changes in flora were observed during lacto bacteriotherapy. Pathogenics ceased to appear, autochthonous micro flora had the following parameters when using the medications
Acidolor -82,7%
Laktoflor – 66,7%
Biosporin– 53,7%
Probiotics are well-tolerated by the patients, demonstrate high clinic result and have a positive effect on the microbiocenosis of the upper air passages.
8. Study of the antagonistic activity of the probiotics, O.Volska, D. Zabolotna , *Journal of ear, nose and throat diseases, №3-c, 2003, p.164-165*
The medications contain both mono cultures of the lactic acid bacilli, and their mixes with various bio-active substances.

Lactic acid bacilli demonstrated wide range of antagonistic activity towards gram-positive, gram-negative and Candida fungi.
At the same time the strains are resistant to most of the antibiotics.
For example, LB3 is resistant to penicillin, benzylpenicillin, oxacillin, ampicillin, cefotaxime, ceftazidime, cefoxitin, cefepime, cefalotin, (cephalosporin antibiotics of 1,2, 3 and 4 generation),
It is also tolerant to fluoroquinolones such as – ciprofloxacin and pefloxacin,
and steroid antibiotics - fusidin.
It is insensitive to lincomycin, cefazolin, azithromycin.
LE strain is resistant to cefalotin, cefotaxime, cefepime, tobramycin, ciprofloxacin, pefloxacin, netilmicin, gentamycin, clindamycin.
It is insensitive to ciprofloxacin, ofloxacin, chloramphenicol.

Thus it enables us to develop strategies of the mixed therapy.