Perspectives of Probiotic Therapy in Sinus Infection

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Introduction: Sinusitis are the nation's primary chronic health problem in USA and many other countries. Sinusitis is a term which refers to inflammation of the lining of the sinuses, called mucosa. Things that can trigger inflammation of the mucosa are a cold, allergies, a deviated septum, reflux disease, nasal polyps and certain chronic diseases. This inflammation can block the narrow passages in the sinuses and prevent mucous from draining properly leading to infection. As a bacterial reservoir, the nose may harbor potentially pathogenic bacteria (PPB) *Streptococcus pneumoniae, Heamophilus influenzae, Staphylococcus aureus, Moraxella cataralis,* ß-hemolytic streptococci and others, including fungal pathogens. In patients carrying PPB, effective antiseptic regimens without negative side effects could be crucial for infection control after major operations on or injuries of the head, nasal sinuses, or lungs. Such regimens may also be important for diabetic patients and persons receiving hemodialysis, in intensive care units, or with impaired immunity due to various other causes.

The causes of sinusitis are numerous and varied. Some of these causes could be treated through avoidance and medications, although for some, surgical treatment may be necessary. The most often used sinus treatments, in the case of an acute, chronic, or recurrent sinus infection, include antibiotics, steroids, antihistamines, mucus thinning agents, decongestants, pain relievers and saline irrigation sprays. Most of these medications may lead to unwanted long-term side effects. Antibiotics, in particular, can have digestive system side effects, which lead to excessive diarrhea. Currently, Probiotics are used to treat sinusitis by increasing the amount of healthy bacteria in the gut, thereby, reducing the amount of diarrhea and other digestive complications. Our aim is to create Target-Specific Probiotic formulations with integrated healing abilities for sinus structures and tissues, high levels of antibacterial activity against specific pathogens, anti-allergy and anti-inflammation activity, the ability to regulate local immune reactions, and have no negative side-effects and complications.

Methods: Previously selected and characterized by FAO/WHO recommendations, strains of *Lactobacillus delbrueckii LE* and *Lactobacillus rhamnosus LB3* as well as in combination (LE+LB3 blend) in the concentration of 5 x 10^9 cells/dose, were under investigation as potential agents for the new ENT products. (FIGURE 1) *In vitro* tests were performed to check the ability to inhibit growth of specific bacterial and fungal pathogens and to assess adhesion properties to nasal epithelial and adenoid cells. *In vivo* trials were conducted on murine models and on humans to evaluate the ability of individual strains and their combinations to demonstrate immune activity, including cytokine and antibody production and regulation, competitive exclusion of pathogens, such as bacteria and fungi, and its efficacy to treat chronic sinus diseases. The immune regulation potency of the probiotic formulations were tested under conditions of immunodeficiency. The immunodeficiency model in mice was designed using a cyclophosphan maneuver in the dose of 50 mg/kg a day before the intake of the probiotic.

Additionally, for the purpose of testing the immune potency of the probiotic formulations, the tonsillar cells from the patients with adenoid disease were cultivated with the medications for 4 hours, at which point the number of Immunoglobulin Fc fragment receptor cells and the antigen CD25 and CD56 natural cytotoxic cells of IgA-producers were tested. In clinical conditions, the preparations were prescribed in one dose (2 ×10^9 - 5 ×10^9) once a day during a ten-day trial. For ablution of the lacunae of the faucial tonsils in patients with chronic tonsillitis and genyantrums in the patients with maxilloeth-moidal sinusitis, the medications were dissolved in 20 ml of normal saline solution. As a control, the existing treatment with antibiotics and antifungal substances were used. Along with the existing treatments, as comparative agents, the probiotic drug, Linex (LEK, Slovenia) and therapeutic yoghurt (Institute Rosell, Canada) were also used.

FIGURE 1

Antagonistic Activity of *Ldelbrueckii LE & L.rhamnosus LB3*

Test-culture	Zones of growth inhibition, mm	
	LE	LB
Escherichia coli M-17	28 ± 1.2	32 ± 2
Enterobacter cloacea	26 ± 1.2	32 ± 1.8
Citrobacter freundii	25 ± 1.1	30 ± 1.1
Escherichia coli k-12	28 ± 1	19 ± 1.2
Kiebsiella pneumoniae K-1	29 ± 1	32 ± 1
Proteus vulgaris 72	12 ± 0.1	27 ± 0.6
Salmonella equiabortus 202	48 ± 3.2	45 ± 3.6
Salmonella typhimurium 11	35 ± 1.3	26 ± 1.9
Serratia marcescens 10	46 ± 3.1	45 ± 3.8
Pseudomonas aeruginose 103	22 ± 1	36 ± 1
Pseudomonas alcaligenes CCM2655	22 ± 1.2	29 ± 1
Micrococcus puogenes	46 ± 2.6	35 ± 3.4
Staphylococcus aureus 209P	33 ± 1.2	31 ± 2.4
Staphylococcus epidermidis	24 ± 1.1	28 ± 2
Candida albicans 212	8 ± 0.6	32 ± 2.4

FIGURE 2

Antagonistic activity of *L.rhamnosus LB3* against pathogens

Test-culture	Antagonism zone , mm	Test-culture	Antagonism zone , mm
Escherichio coli	42.3 ± 0.3	*Peptococcus niger*	15.1 ± 2.5
Enterobacter Clocae	32.2 ± 0.3	*Peptostreptococcuc anaerobius*	20.2 ± 1.5
Citrobacter freundii	30.1 ± 1.5	*Bacteroides fragilis*	10.0 ± 2.5
Klebsiella pneumoniae	30.3 ± 1.2	*Bacteroides melaninogenica*	10.3 ± 1.5
Pseudomonas ueruginosa	32.0 ± 2.0	*Actnomyces israeli*	0
Micrococcus luteus	30.2 ± 0.2	*Streptomyces flava*	20.3 ± 0.9
Staphylococcus aureus	38.4 ± 1.42	*Candida albicans*	28.2 ± 1.8
Staphylococcus epidermidis	25.9 ± 2.4	*Candida tropicalis*	30.4 ± 2.0
Streptococcus viridans	28.5 ± 0.4	*Candida krusei*	20.6 ± 2.1
Enterococcus faecalis	30.6 ± 1.4	*A.colcoaceticus*	35.3 ± 0.75
P.fluorescens	30.4 ± 1.0	*Serratia marcescens*	36.4 ± 1.12
Bacillus.linens	30.7 ± 1.09	*Bacillus.mycoides*	30.5 ± 0.95
Bacillus.megaterium	33.2 ± 1.21	*Bacillus.subtilis*	34.1.5 ± 1.18
Bacillus.cereus	37.7 ± 2.07	*Staphylococcus citreus*	37.5 ± 1.17
Nocardia carollina	22.3 ± 1.16		

FIGURE 3

LABs and pathogens adhesion to human cells

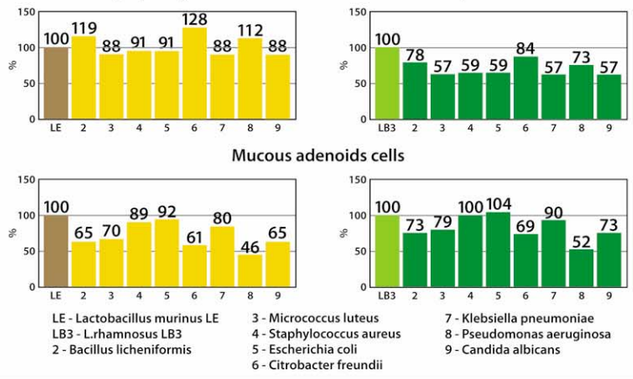

FIGURE 4

Probiotic doses with Fungicidal and Fungiostatic activity against Candida pathogens

Type of activity	LE	LB3	LE + LB3
Fungicidal (kill) activity	5.0 X 10^9	1.25 X 10^9	0.5 X 10^8
Fungiostatic activity	2.5 X 10^9	6.25 X 10^8	2.5 X 10^7

FIGURE 5

Immune activity of the patient's palatil tonsil cells after caltivation with probiotic cells LB3 and LE

Type of activity	NK cells		IgA-C, %	
	average	min-max	FcR	IgA-e
Control (n=10)	8.7	6 - 20	15.2	14.7
LB3 (n=9)	23.5*	6 - 75	20.3*	19.6*
LE (n=9)	30.1*	8 - 100	20.2*	15.1
Jougurt (Canada)	11.5	6 - 17	–	–

*p<0.05

It was reported that application of the formulation based on L. *rhamnosus LB3* resulted in the statistically reliable increase of the number of tonsillar cells producing IgA as well as *L.delbrueckii LE* increased activity of the natural cytotoxic tonsillar cells against xenoerythrocytes. (FIGURE 5)

Formulation based on *L. deldrueckii LE* increased the number of tonsillar cells with surface antigens CD25 and CD56. (FIGURE 6)

Results: Researched probiotics demonstrated high levels of antagonistic activity towards the most frequent microbes found during recrudescence of inveterate lemic-inflammatory diseases of ENT organs, (FIGURE 2) showed high degree of conglutination to mucous coat of the upper air passages, (FIGURE 3) and demonstrated acid and alkaline tolerance. Furthermore, *L.rhamnosus LB3* was able to suppress growth of 90% of clinical Candida strains, including *C.albicans,C.krusei, C.tropicalis,* etc. (FIGURE 4)

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Researched probiotic formulations induced formation of valid immune response by TH1 type, also inhibited fatty cellular infiltration of the tonsils tissue, reduced the risk of the inflammatory edema and stimulated progression of B-cell lymphocytes and high glycogen macrophages. They also have shown inhibitory influence on the non lymphoid elements, connected with progression of the allergic inflammations.

It was observed that probiotic formulations stimulated IFN up to 4.5-fold, induced production of the IL- 4, increased IgG and IgA up to 2.5 fold, and intensified glycogen synthesis in phagocytes. *L. rhamnosis LB3* demonstrated more effective activation of humoral immune response; whereas, *L.delbrueckii LE* showed mostly cell-mediated immune response. (FIGURES 7 & 8)

In clinical conditions, combination of LB3 and LE demonstrated positive results in 85.5% cases of the chronic tonsillitis and 90% in maxilloethmoidal sinusitis as compared to positive results in 72.8% cases in the control patient group using antibiotics and antifungal substances. Performance capability was increased in 100% cases, the physiologic flora was restored, the amount of the pathogenic and opportunistic flora was reduced in 4-6 times, enzymatic activity was restored to the normal level and immune parameters were normalized. Probiotics were well-tolerated by the patients, demonstrated high clinical result and have had a positive effect on the microflora of the upper air passages. The comparative agents of Linex and yoghurt were not statistically effective.

Conclusions: The new probiotic strains of *L.delbrueckii LE* and *L.rhamnosus LB3* were deeply investigated and demonstrated their marked capacities for use in the development of target specific probiotic products for ENT infection and inflammation diseases. (FIGURE 9) The results also indicated that specific probiotic products and probiotic therapy treatment can reduce PPB in the upper respiratory tract as well as eliminate or reduce possible post treatment negative side effects and/or complications.Therefore, probiotic formulations based on LB3 and LE strains proved to be more efficient and active compared to the existing treatment and the comparative agents.

FIGURE 6

Population of CD25+ cells and CD56+ cells in patient's palate tonsil cells after caltivation with probiotic cells LB3 and LE

Type of activity	Population of CD25+ cells, %		Population of CD56+ cells, %	
	average	min-max	average	min-max
Control (n=10)	12.3	3-17	15.2	6-30
LB3 (n=9)	14.4	6-30	16.1	7-28
LE (n=9)	23.4*	8-50	21.7*	10-35

*p<0.05

FIGURE 7

IFN Activity Induced by Live Probiotic Cells LB3 and LE

Product/concentration		IFN titer, unit/ml Observation time, hours 24	48
Control		<64	<64
L. rhamnosus LB3	5 X 10^6 cfu/ml	64	32
L. rhamnosus LB3	5 X 10^5 cfu/ml	64	16
L. delbrueckii LE	5 X 10^6 cfu/ml	128	64
L. delbrueckii LE	5 X 10^5 cfu/ml	128	32

FIGURE 8

Influence of different age cultural medium for co-induction of IFN a/β by human tonsil lymphocytes

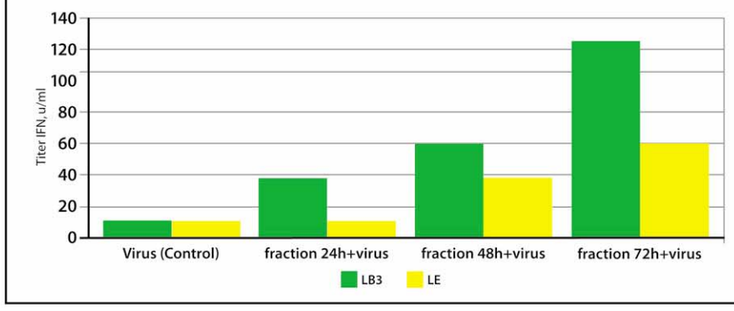

FIGURE 9

Probiotic properties of the new Lactobacillus strains

Strain	pH Stability	Adhesion	Antagonistic activity against				°T	Proteolytic Activity u/ml	Antimutagenic Activity %	Macrophages Activity % / NK % cells
			Gr-	Gr+	Yeasts	Candida				
LB3	1.5 - 8.5	5.06 ± 0.23	35.8 ± 0.8	34.5 ± 1.3	21.0 ± 1.2	26.4 ± 1.9	210.0 ± 4.5	637.0 ± 1.7	99.03 ± 2.9	163/270
LE	1.5 - 8.5	4.93 ± 0.17	31.2 ± 0.9	30.1 ± 1.1	32.6 ± 1.2	11.2 ± 0.8	170.0 ± 7.8	74.82 ± 2.3	90.38 ± 3.1	122/345

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