Original Research Article

EFFECTS OF PROBIOTICS ON INTESTINAL MICROFLORA OF HIV-INFECTED INDIVIDUALS

ABSTRACT

Aims: HIV-1 infection results in structural damage to the intestinal mucosa and changes of gut microflora following dysfunction of the gastrointestinal system, including compromised barrier function. Known properties of probiotics suggest that they may be useful tools in restoring normal intestinal flora. Our study goal was to determine whether the use of a probiotics can recover normal gut flora in chronically HIV-infected adults.
Study design: Cohort Design
Place and Duration of Study: Sumy State University, Medical Institute. Department of Microbiology and Clinical Immunology
Methodology: The study involved 40 HIV-1-infected patients of the regional center of prevention and control of AIDS in Kharkov. All the patients were informed about the purpose and plan of study and gave their written agreement to participate in the study. All the patients had been diagnosed according to the criteria of WHO with the III-IV stage of HIV infection. During the month before the survey the patients did not take any antibiotics. Dysbiosis correction circuit was designed for one month taking of probiotic preparations. Six weeks later the follow-up study was conducted to investigate gut microflora of 20 HIV-infected patients.
Results: Changes of intestinal microbiota were found in all of the patients. In the most cases the decrease of obligatory microorganisms, especially *Bifidobacterium* spp. (in 90 % of patients) was found. Overgrowth of major opportunistic pathogens (*S. aureus* and *Candida spp*.) was registered in only a minority of patients. The probiotic interventions resulted in significantly elevated levels of beneficial bacteria load (such as *Bifidobacterium spp*, *Lactobacillus spp*.) and a decrease in patogenic bacteria load (such as *Clostridium spp, Candida spp*).
Conclusion: Probiotic preparations can successfully augment the levels of beneficial species in the gut during chronic HIV-1 infection. These findings may help inform future studies aimed at testing pre- and probiotic approaches to improve gut function and mucosal immunity in chronic HIV-1 infection.

Keywords: microbial translocation; inflammation; probiotic bacteria; lactobacillus; HIV-1; anti-retroviral therapy (ART).

1. INTRODUCTION

It is known that the total number of microorganisms inhabit the human gut (1014), on two orders exceeds total number of the cells. Besides, a large number of exogenous xenobiotic including pathobionts and food antigens passes through the intestine daily. It's not a surprise that up to 80 % of the lymphoid tissue is associated with the intestine region (GALT).

Indigenous intestinal microflora has a symbiotic relationship with the intestinal mucosa and is an integral part of the gastrointestinal tract. Close interaction between the microbiota and mucosa is a major imperative of intestinal homeostasis [6, 12]. It has been found out recently that dysbiotic changes in the gut (dysbiosis) accompany not only various intestinal disorders, but are also associated with a wide range of multi-organ pathologies, including HIV infection [5, 19].

It is shown that after penetrating into the mucosa HIV infects 60% of resting Ki67-CD4+ T-cells, leading to their activation. Activated CD4 + T-cells actively produce virus which infects cells via the neighbor cell contacts and circulates through the bloodstream to distant organs and tissues. As a result, the body forms a large reservoir of active infectious virus that can't be neutralized so far. [18]

Equally important is the direct impact of the virus on enterocytes. HIV has been established to infect and destroy vast amount of GALT CD4+ T cells and dendritic cells, as well as affect directly enterocytes: tat protein of HIV inhibits glucose uptake by enterocytes, impairing their function, gp120 protein increases the amount of calcium in the cells, which causes depolymerization of tubulin and, consequently, dysfunction of cytoskeleton. This leads to disruption of intercellular interaction and increased permeability of the intestinal barrier. At the same time the expression of genes that control the integrity of epithelium is suppressed [9].

Enteropathy accompanied by HIV is characterized by villous atrophy, crypt hyperplasia, malabsorption of several important nutrients, apoptosis of enterocytes, and increased permeability of epithelium. Mass deaths of the immune effector cells in the lamina propria, destruction of Peyer's patches, and a sharp reduction of secretory IgA and defensins levels create favorable conditions for the breeding of excessive microflora including pathogenic one in the intestinal lumen [14].

These factors lead to the penetration of LPS and other bacterial components through the intestinal barrier into the blood circulation although bacteremia is not observed as a rule. Translocation of LPS and chronic exposure to peripheral lymphocytes result in persistent systemic immune response accompanied by high level of proinflammatory cytokines, which fairly soon leads to the depletion of the immune system. It is believed that translocations and chronic immune activation play a key role in the development and progress of opportunistic complications [1,11].

Although it is not clear whether the dysbiosis of intestinal microbiota in HIV infection is a primary factor leading to the development of the disease or secondary response to other factors; but it is evident that it plays a significant role in the chronic phase of infection and the appearance of opportunistic complications.

This opens up the prospect of influence on the infectious process by correcting dysbiotic changes in HIV-infected patients. In this regard, the aim of the present study was to evaluate changes in microflora of the large intestine in chronic HIV infection and the possibility of correction by means of bacterial preparations (probiotics).

2. MATERIAL AND METHODS / EXPERIMENTAL DETAILS / METHODOLOGY

2.1 Patients

The study involved 40 HIV-1-infected patients of the regional center of prevention and control of AIDS in Kharkov. All the patients were informed about the purpose and plan of study and gave their written agreement to participate in the study. All the patients had been diagnosed according to the criteria of WHO with the III-IV stage of HIV infection.

Patients were divided into the two groups:

I – n=28 (70%) patients, the number of CD4 + cells at the time of the study was lower than <350 cells / µl.

II – n=16, patients, the number of CD4 + cells at the time of the study was higher than <350 cells / µl.

During the month before the survey the patients did not take any antibiotics. Dysbiosis correction circuit was designed for one month taking of probiotic preparations. Six weeks later the follow-up study was conducted to investigate gut microflora of 20 HIV-infected patients.

2.2 Fecal Bacteriologic Culture

The contents of the colon in an amount of 2-3 g was taken to the laboratory and processed within 2 hours in a sterile vial without preservative. Collection of material was carried out before the use of antibiotics and bacterial preparations (probiotics, prebiotics et al.).

Primary inoculation of clinical material was performed quantitative method on nutrient media in accordance with the regulations. Ten-fold serial dilutions of each fecal sample were performed and plated on selective and non-selective media for enumeration of the members of the intestinal microflora. Stool samples were placed on solid media (Bismuth Sulphite Agar, EMB Agar (Levine), Endo Agar, Blood Agar, Baird-Parker Agar, Sabouraud Dextrose Agar, Clostridial Agar, Rogosa SL Agar, Bifidobacterium Agar, HiMedia Lab., India). The plates were incubated at 37 'C for 24 or for 48 h. The incubated microorganizms were then counted and identified with accordance to standard procedures. Summarized data of healthy adults microflora contents served as a normal standard. All bacterial counts (colony-forming units (CFU)/g of wet feces) were transformed to logarithm (log_{10}CFU) for ease of statistical analysis.

2.3 Statistical Analysis

The results are presented in the form of averages, standard deviation and median assuming a normal distribution of data. Normal distribution of quantitative traits was verified by the Shapiro-Wilk test. The research results are processed using "STATISTICA 10.0" (StatSoft Inc., USA, version 10.0.1011.6) and spreadsheet editor Microsoft Excel 2013.

3. RESULTS AND DISCUSSION

27 of 40 (67.5%) HIV-infected patients participated in the study were women and 13 (32.5%) men. The average age of patients was 35.6 ± 8.2 years. The average number (M ± m) of CD4+ T cells before the study was 426 ± 264 in 1 µl (Table 1).

Table 1. Patients characteristics of the study groups (n=40)

Characteristic	Value
Age ((year) *M±SD*;* *Median*))	 36.6±8.2 34
Sex n/% -male -female	 13/32.5 27/67.5
Blood CD4 cell count (cells/ml)	
- all(n=40) *M±SD;* *median*	 426±264 416
- I group	223±99 220
- II group	462±280 424

**Mean ± standard deviation (SD)*

Table 2. Composition of gut flora in HIV-1 infected patients before the correction of probiotic preparations

Covariates	Concentration of bacteria log_{10}CFU	Patients (n=40)	Normal
Bifidobacterium spp.	5.9 ± 0.9 7.0 ± 1.1	36 4	9.7 ± 1.4
Lactobacillus spp.	5.0 ± 0.8 6.7 ± 1.07	35 5	7.7 ± 1.23
E. coli (lac+)	5.9 ± 1.2 8.7 ± 1.4	24 16	8.0 ± 1.3

E. faecalis	5.0 ± 1.02 7.7 ± 0.8	25 15	7.74 ± 1.2
E. faecium	5.0 ± 0.8 5.9 ± 0.9	34 6	7.7 ± 1.2
E. coli Hly	ND 5.0 ± 0.8	2 38	ND
S.aureus	ND 4.0 ± 1.6	33 7	ND
S. epidermidis	4.0 ± 0.8 5.0 ± 1.4	27 13	4.0 ± 0.6
Candida spp.	2.9 ± 0.5 4.0 ± 1.5	32 8	4.0 ± 0.6
Cl. perfringens	2.0 ± 0.3 2.9 ± 0.4	38 2	2.9 ± 0.5

Data as mean ± standard deviation (Log_{10}counts/g feces)
ND not detected

As it can be seen from *Table 2*, the quantitative and qualitative composition of the normal microflora of the large intestine has been broken in all patients.

Changes are identified among anaerobic and facultative anaerobic bacteria. It should be noted that violations of microbiota not followed emerging opportunistic pathogens. Reduced number of organisms concerned primarily *bifidobacteria,* which dominated in the anaerobic flora and accounted for about 95% of the intestinal microbiota. According to our data, in 90 % of cases the number of *bifidobacteria* was less than 5.9 ± 0.9 and in 10 % of cases it was about 7.0 ± 1.1. The number of very important *lactobacilli* at HIV infection is significantly reduced against healthy controls ([14]) accounting less than 5.0 ± 0.8 in 87.5 % and 6.7 ± 1.07 in 12.5 % of patients against 7.7 ± 1.23 respectively ($p<0.05$).

The group of anaerobic bacteria, *Bacteroides*, wasn't detected in patients. The leading representative of the facultative anaerobic bacteria belonging to the indigenous microflora is *E. coli*. The number of bacteria of this type in about half of virus carriers remained constant while the other was reduced by 1-2 orders of magnitude. Hemolytic *E. coli* strains in small concentrations detected in only 5% investigated.

The same trend is observed in relation to other pathobionts: *S. aureus, S. epidermidis,* and *C. albicans* in low titers are found in only a minority of infected patients ($p<0.05$). Only in one patient *Clostridium spp.* were isolated in very low concentrations. In addition, a case of a serious intestinal dysbiosis in HIV-infected patients was accompanied by falling down on 1-2 orders of the obligate commensals *E. faecalis,* and *E. faecium* presented in large numbers in the faeces of healthy adults.

Thus, HIV infection, regardless of the duration of the course, the clinical stage of the disease and antiviral managing manifests a profound violation of the gut homeostasis accompanied by a simultaneous decrease in quantitative anaerobic (*bifidobacteria* and *lactobacilli*) and facultative anaerobic flora (*E. coli*).

Probiotic preparations used for the dysbiosis correction contained such strains of microorganisms as:

1. *Lactobacillus casei, L. rhamnosus, L. acidophylus, L. bulgaricus, Streptococcus thermophylus, Bifidobacterium breve, B. longum*
2. *Bifidobacterium bifidum, Escherichia coli (stain M-17)*
3. *Saccharomyces boulardii*

In our study, the use of probiotic bacterial preparations on the background of the microbiome dysbiosis in HIV-infected patients resulted in a significant mitigation of these violations, but complete restoration was not also observed (*Table 3*).

Table 3. Composition of gut flora in HIV-1 infected patients after the correction of probiotics

Covariates	Concentration of bacteria log_{10}CFU	Patients (n=20)	Normal
Bifidobacterium spp.	5.9 ± 1.4 8.0 ± 1.8	19 1	9.7 ± 1.4
Lactobacillus spp.	5.0 ± 1.1 7.7 ± 1.7	12 8	7.7 ± 1.23
E. coli (lac+)	8.7 ± 1.9	20	8.0 ± 1.3
E. faecalis	5.0 ± 1.1 7.7 ± 1.7	6 14	7.74 ± 1.2
E. faecium	5.0 ± 0.9	20	7.7 ± 1.2
E. coli Hly	ND 5.0 ± 1.1	19 1	ND
S.aureus	ND	20	ND
S. epidermidis	ND	20	4.0 ± 0.6
Candida spp.	ND	20	4.0 ± 0.6
Cl. perfringens	2.0 ± 0.5 2.9 ± 0.7	16 4	2.9 ± 0.5

Serious changes of the intestine microflora in chronic HIV infection have been identified by other researchers, too. Deep changes of intestinal microbiota is accompanied by the appearance of communities of enteropathogenic bacteria capable of converting tryptophan to kynurenine immunomodulatory derivatives, which correlates with the progression of the disease and contributes to the violation of mucosal immunity. At the same time ART-naïve patients increases the levels of some bacterial taxa, and the suppression of 45 taxa. The most significant enrichment was mentioned for Erysipelotrichaceae, which often accompanies obesity and is associated with increased incidence of cardiovascular system disorders. Such types as *Proteobacteria* are part of the most enriched genera of ART-naïve patients. Among them is the species included in the genera of *Salmonella, Escherichia, Serratia, Shigella* and *Klebsiella* of the *Enterobacteriaceae* family, known as pro-inflammatory pathobionts. The gut content of ART-naÏve HIV-carriers is enriched with *Staphylococcus, Pseudomonas, Campylobacter spp., Candida albicans*, which often cause opportunistic infections and bacteremia, with a significant decrease in the content of *bifidobacteria* and *lactobacilli, Clostridia* and *Bacteroides* with particularly strong suppression of *Bacteroides* and *Alistipes* genera. The studies showed the dramatic reducing the levels of lacto-and *bifidobacteria* and increasing the concentration of pathogenic species, including *Candida albicans* and *Pseudomonas aeruginosa* in HIV-carries [2, 7]. Such probiotics as *Lactobacillus rhamnosus* GR-1 have a beneficial effect on preservation of immunity in HIV infection [4].
Recently, it has been found that the balance between the two subpopulations of CD4 + regulatory T cells, Th17 and CD25 + FoxP3 + (Treg) is responsible immune mechanisms which protect against infections and autoimmune disorders.

Treg-cells express toll-like receptor 4 (TLR-4) and activated by LPS. Some *Lactobacillus* species (*L. reuteri* and *L. casei,* but not *L. plantarum*) also activate these cells [15]. The number of HIV-specific Treg-cells is increased in patients responding to ART.

While deep depletion of Th17-cells and reduction of CD4+ CD161+ cells associated with progressive loss of Treg-cells, increased immune activation and progression of the disease [13]. The combination of probiotics in the model system can increase the content of Treg-cells, and suppress the development of the disease [10]. Activity had only a mixture of several species of *Lactobacilli*. Suppressive activity was accompanied by increased secretion of IL-10 Treg-cells, which led to a weakening of the secretion of pro-inflammatory cytokines by cells Th1 and Th17. Model system showed that taking of probiotics (*L. acidophilus, L. casei, L. reuteri, Bifidobacterium bifidium* and *Streptococcus thermophilus*) induced a low response of T and B cells, reduced the secretion of Th1, Th2 and Th17 cytokines, inhibited apoptosis and caused migration of Treg-cell into the inflammation area [3]. Probiotics have a beneficial effect on the HIV-infection. [4]. Gori et al. have shown that simultaneous use of probiotics results in a significantly increased number of *bifidobacteria* and reduction of *Clostridium coccoides, Eubacterium rectale, Clostridium lituseburense* and *Clostridium histolyticum* [3]. It is also shown that the oligosaccharide mixture as a prebiotic reduces the level of sCD14, decreases activation of CD4+ T-cells and enhances the NK-cell activity in ART-naïve HIV-infected adults. [8].

Thus, the mechanism of probiotic bacteria effect on the immune status and HIV infection may influence on the translocation and activation of the immune balance of regulatory T-cells. These findings thus suggest that the correction of dysbiosis can have desirable effects in the restoration of intestinal function and repair.

4. CONCLUSION

In conclusion, a decrease in total obligate anaerobes and an increase in pathogenic bacteria in the gut are indicated in patients with HIV-1 and probiotic preparations can successfully augment the levels of beneficial species in the gut. These findings may help inform future studies aimed at testing pre- and probiotic approaches to improve gut function and mucosal immunity in chronic HIV-1 infection.

REFERENCES

1. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12:1365–71.

2. Das JK, Mishra D, Ray P, Tripathy P, Beuria TK, et al. In vitro evaluation of anti-infective activity of a Lactobacillus plantarum strain against Salmonella enterica serovar Enteritidis. Gut Pathog. 2013;5: 11. doi: 10.1186/1757-4749-5-11.

3. De Roock S.; van Elk M.; van Dijk M.E.; Timmerman, H.M.; Rijkers, G.T.; Prakken, B.J.; Hoekstra, M.O.; de Kleer, I.M. Lactic acid bacteria differ in their ability to induce functional regulatory T cells in humans. Clin. Exp Allergy. 2010;40:103–110.

4. Hemsworth J, Hekmat S, Reid G. The development of micronutrient supplemented probiotic yogurt for people living with HIV: Laboratory testing and sensory evaluation. Innov Food Sci Emerg Technol. 2011; 12: 79–84.

5. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, Strowig T, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature. 2012; 482:179–85. doi:10.1038/nature10809nature10809.

6. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol. 2010;10:159–69.doi:10.1038/nri2710.

7. Gori A, Tincati C, Rizzardini G, Torti C, Quirino T, Haarman M, Ben Amor K, van Schaik J, Vriesema A, Knol J, et al. Early impairment of gut function and gut flora supporting a role for

alteration of gastrointestinal mucosa in human immunodeficiency virus pathogenesis. J. Clin. Microbiol. 2008;46:757–758.

8. Gori, A, Rizzardini G, Van'T Land B, Amor K.B, van Schaik J, Torti C, Bandera A, Knol J, et al. Specific prebiotics modulate gut microbiota and immune activation in HAART-naive HIV-infected adults: Results of the "COPA" pilot randomized trial. Mucosal Immunol. 2011;4:554-563.

9. Guadalupe M, Sankaran S, George MD, Reay E, Verhoeven D, Shacklett BL, et al: Viral suppression and immune restoration in the gastrointestinal mucosa of human immunodeficiency virus type 1-infected patients initiating therapy during primary or chronic infection. J Virol. 2006;80 (16):8236–8247.

10. Lavasani S, Dzhambazov B, Nouri M, Fak F, Buske S, et al. A novel probiotic mixture exerts a therapeutic effect on experimental autoimmune encephalomyelitis mediated by IL-10 producing regulatory T cells. PLoS One. 2010; 5: doi: 10.1371 / journal.pone.0009009

11. Mehandru S, Poles MA, Tenner-Racz K, et al. Mechanisms of gastrointestinal CD4+ T-cell depletion during acute and early human immunodeficiency virus type 1 infection. J Virol. 2007;81:599–612.

12. Pabst R, Russell MW, Brandtzaeg P. Tissue distribution of lymphocytes and plasma cells and the role of the gut. Trends Immunol. 2008;29:206–8. doi:10.1016/j.it.2008.02.006.

13. Prendergast A, Prado JG, Kang YH, Chen F, et al. HIV-1 infection is characterized by profound depletion of CD161 + Th17 cells and gradual decline in regulatory T cells. Aids. 2010;24:491-502.

14. Saxena D, Li Y, Yang L. et al. Human Microbiome and HIV/AIDS // Curr HIV/AIDS Rep. 2012;9(1):44–51. doi:10.1007/s11904-011-0103-7

15. Smits HH, Engering A, van der Kleij D, de Jong EC, Schipper K, et al. Selective probiotic bacteria induce IL-10-producing regulatory T cells in vitro by modulating dendritic cell function through dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin. J. Allergy Clin. Immunol. 2005;115:1260-1267.

16. The principles of correction of disorder of microecology of intestine: methodic recommendations / Moscow Research Institute of Pediatrics and Pediatric Surgery. – Moscow. 2004;38.

17. The current approaches of the correction of intestinal dysbiosis: methodic recommendations / Ukrainian Centre for Scientific Medical Information and patent-license work. 2000;27.

18. Turville SG, Peretti S, Pope M: Lymphocyte-dendritic cell interactions and mucosal acquisition of SIV/HIV infection. Curr Opin HIV AIDS. 2006;1(1):3–9.

19. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci. Transl. Med. 2013; 5: doi:10.1126/scitranslmed.3006438