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Improvement of neuropsychological performances and reduction of immune-activation markers after probiotic supplementation and change of life-style in an HIV positive male: targeting the microbiota to act on gut-brain axis

G. Ceccarelli[1], P. Vassalini[1], G. Corano Scheri[1], E. N. Cavallari[1], L. Bianchi[1], G. Di Girolamo[1], M. Fratino[2], V. Vullo[1], G. D'Ettorre[1]

[1]Department of Public Health and Infectious Diseases, Sapienza University of Rome, Rome, Italy
[2]Department of Neurology, Sapienza University of Rome, Rome, Italy

ABSTRACT:

The gut-brain axis is widely influenced by the intestinal microbiota and dysbiosis is consequently associated with a large dysregulation of its functions. Probiotic supplementation, reducing the harmful effects of dysbiosis, has shown positive effects not only on gut and brain functions, but also on the control of the dangerous effects of immune activation. Mounting evidence has shown that neurocognitive impairment can be a secondary to the impairment of the microbiota-gut-brain axis in HIV positive patients. In this case report we analyzed the improvement of neurocognitive performances associated with a reduction of levels of peripheral immune-activation, after 6 months of probiotic supplementation. In this case, the achieved result may have been influenced by a more comprehensive modification of the patient's lifestyle with the introduction of a controlled diet and regular physical activity. Our observations suggest that integrate antiretroviral therapy and non-pharmacological tools into an overall approach, can be a useful strategy to control some non-AIDS related diseases.

Keywords: Neuropsychological tests, Gut-Brain axis, Central Nervous System, Cerebrospinal fluid, CD4+, CD8+, CD38+, HLA-DR+, HIV, Probiotics.

List of Abbreviations: HIV: Human Immunodeficiency Virus; MSM: Man who have sex with men; CSF: Cerebrospinal fluid; GALT: gut-associated lymphoid tissue; IDO: indolamine-2,3-dioxygenase; hsCRP: high sensitivity C-Reactive Protein; LBP: lipopolysaccharide binding protein; GALT: gut-associated lymphoid tissue.

INTRODUCTION

HIV infection is a chronic inflammatory disease in which several immunological and functional disorders, associated to a pro-inflammatory change in the gut microflora, are described[1].

Recent advances in medical research have reported the importance of intestinal microbiota in influencing "the gut-brain axis" (GBA), linking cognitive and emotional centers of the brain with peripheral enteric nervous system and intestinal functions[2]. Neural and endocrine networks, immune and humoral links ap-

Corresponding Author: Giancarlo Ceccarelli, MD; e-mail: giancarlo.ceccarelli@uniroma1.it

pear to affect this bidirectional interaction between microbiota and GBA[2]. In particular, the gut microbiota is involved in the regulation of cognition, mood and anxiety and can impact cognitive and mood functions in different ways, i.e. by releasing bacterial metabolites or producing neuroactive substances[3-5].

Probiotic supplementation has shown positive effects on the control of the dysbiosis associated to many chronic inflammatory bowel conditions and diseases[6]. Moreover, recent studies conducted in HIV population have reported that probiotic supplementation was associated to I) a recovery of the integrity of the gut epithelial barrier; II) a reduction of intraepithelial lymphocytes and enterocyte apoptosis; III) a reduction in the levels of immune activation on CD4 T-lymphocytes, for both markers CD38 and HLA-DR, high sensitivity C-Reactive Protein (hs-CRP) and lipopolysaccharide binding protein (LBP) plasma levels; IV) a significant reduction of neopterin in cerebrospinal fluid (CSF) and indolamine-2,3-dioxygenase (IDO) mRNA levels in gut-associated lymphoid tissue (GALT); V) an improvement in same neurocognitive functions[7-10]. Despite these preliminary observations, more evidence is needed to better understand the contribution of the probiotics in modulating Gut-Brain Axis in HIV patients. In the same way, interesting studies have showed that also a regular physical activity may result in I) a decrease in chronic inflammation and related immune activation; II) a concomitant reduction in the damages related to non-AIDS defining illnesses; III) an improvement in overall neuropsychological function[11]. Considering the importance of this preliminary evidence, several trials are ongoing to accurately assess these data[12-18].

CASE REPORT

Hereby we present the case of a 56-year-old Caucasian man, who has sex with men (MSM), diagnosed for HIV infection in 1989. The Patient signed both written informed consent and consent for publication of anonymized data. He was lost to follow up since 1994, when he was admitted to our Hospital because of with *Pneumocisis jirovecii* pneumonia and atypical mycobacterial infection, with a nadir of $CD4^+$ count of 1/mmc (CDC AIDS Score: C3). He underwent various antiretroviral regimens with repeated treatment failures due initially to the poor adherence, and after to the side effects of therapy (i.e. peripheral neuropathy, lipodystrophy and dyslipidemia). For these reasons, he changed several antiretroviral treatments as reported in Table 1. The current combined antiretroviral therapy (cART), started in May 2015, is Dolutegravir, Darunavir/r, Saquinavir and Emtricitabine.

The immune-virologic control was obtained in 2011 with persistent undetectable HIV viral load and stable recover of lymphocytes T-$CD4^+$ >350 cell/mmc. The levels of immune activation on $CD4^+$ and $CD8^+$ T-lymphocytes, for both markers CD38 and HLA-DR and their simultaneous expression, recorded at April 2016, were showed in Table 2.

Table 1. History of antiretroviral treatments.

1995-1997	ZIDOVUDINE + DIDANOSINE
1997-1997	SAQUINAVIR + LAMIVUDINE + STAVUDINE
1999-2003	EFAVIRENZ + LAMIVUDINE + ZIDOVUDINE
2003-2004	ATAZANAVIR/r + SAQUINAVIR + TENOFOVIR
2004-2007	TIPRANAVIR/r + ABACAVIR + TENOFOVIR
2007-2008	TENOFOVIR + EMTRICITABINE + DARUNAVIR/r
2008-2012	LOPINAVIR/r + TENOFOVIR+ ENFUVIRTIDE + LAMIVUDINE + ZIDOVUDINE + RALTEGRAVIR
2012-2013	TENOFOVIR + EMTRICITABINE + RALTEGRAVIR + EFAVIRENZ
2013-2014	DARUNAVIR/r + ETRAVIRINE
2014-2015	DARUNAVIR/r + SAQUINAVIR + EMTRICITABINE
2015-today	DOLUTEGRAVIR + DARUNAVIR/r + SAQUINAVIR + EMTRICITABINE

Other relevant conditions in his medical history were osteopenia/osteoporosis and a partial impairment of neurocognitive performance without severe reductions in working capacity, evidenced from 2015 (Table 3).

On June 2016, the patient spontaneously started a healthy lifestyle based on a change in eating habits and on the abandonment of physical inactivity. A Mediterranean diet with restricted caloric intake was adopted and a probiotic supplementation with daily dose of 2 sachets was started. The multistrain probiotic used contained Bifidobacteria (B. breve DSM 24732, B. longum DSM 24736, B. infantis DSM 24737), Lactobacilli (L. acidophilus DSM 24735, L. plantarum DSM 24730, L. paracasei DSM 24733, L. delbrueckii subsp. bulgaricus DSM 24734) and Streptococcus thermophilus DSM 24731.

At the same time, he began a regular physical activity, and gradually came to make jogging twice a week for about 40 minutes each time, and swimming for 60 minutes one time, weekly.

Six months after the start of this healthy lifestyle, the patient underwent to a clinical, neuropsychological and viro-immunological follow-up. As reported in table 2, a reduction peripheral in immune-activation was observed. Moreover, HIV-RNA remained undetectable and the number of $CD4^+$ increased from 411 to 532 cells/mmc. Also the metabolic parameters were improved with better control of the dyslipidemia previous reported.

The evaluation of neuropsychological assessment was performed through a complete neuropsychological test battery (Mini-Mental State Examination followed by specific tests exploring the main cognitive domains such as memory, attention, executive function, language). A parallel form of RAVLT (immediate and delayed recall) was used, according to the Italian validation. At the same time, to assess psychological manifestations (alexithymia, depression, state and trait anxiety) the patient was evaluated through a set of questionnaires including State-Trait Anxiety Inventory Y-1 and Y-2, Toronto Alexithymia Scale-20 and Beck Depression Scale II[18-31]. When

Table 2. Changes in immune-activation levels before and after 6 months of probiotic supplementation.

	$CD4^+$ %	$CD4^+$ $CD38^+$ %	$CD4^+$ $HLA\text{-}DR^+$ %	$CD4^+$ $CD38^+$ $HLA\text{-}DR^+$ %
T0	17.70	13.76	6.52	2.31
T6	31.25	2.48	3.47	0.32
	$CD8^+$ %	$CD8^+$ $CD38^+$ %	$CD8^+$ $HLA\text{-}DR^+$ %	$CD8^+$ $CD38^+$ $HLA\text{-}DR^+$ %
T0	70.38	6.20	9.60	4.38
T6	64.65	0.49	3.28	0.79

neurocognitive data were analyzed, a significant difference between T0 and T6 was observed: in fact, most of test scores increased significantly as showed in table 3. By contrast, no significant differences were observed for FAB (Frontal Assessment Battery), TMTA (Trail Making Test A), Verbal Span-Digit Forward, Verbal Span-Digit Back, BDI II (Beck Depression Scale), AAT (Aachen Aphasia Test), CBTT- Forward (Corsi block tapping task), CBTT-Backward (Corsi block tapping task) examined before and after probiotic intake.

The increase of fecal Bifidobacteria spp, compared to their basal level, confirmed the adherence to the probiotic supplementation.

DISCUSSION

Neurocognitive disorders are commonly reported in cART treated HIV patients[32]. Recently, pivotal studies have increased the understanding of the role played by the GBA in neurocognitive disorders. These data indicate that alterations in microbiota quality and amount can substantially affect the brain physiology, and differential microbial composition is associated with alterations in behavior and cognition[33-35]. Studies exploring the effects of probiotics on gut microbiota composition have shown that supplementation can play a protective role on modification of mood and behavior, worsening of cognition related to alterations of microbioma and to dysbiosis[36-38]. In our report, the patient regularly took a high concentration multi-strain probiotic powder supplement twice a day for six months as documented by the increase of fecal Bifidobacteria spp, compared to their basal level.

After six months of probiotic intake, our patient presented a significant improvement of his performance in the most of neuropsychological and behavioral tests. The possible effect of probiotic supplementation on neurocognitive performances could be linked to different pathophysiologic mechanisms: in fact, experimental and clinical evidence suggest that gut microbiota inter-

Table 3. Results of neuropsychological test administered before and after 6 months of probiotic supplementation.

	Results at T0	Results at T6
MMSE (Mini Mental State Examination)	25	30
RAVLT (Rey Auditory Verbal Learning Test Immediate Recall)	29	53
RAVLT (Rey Auditory Verbal Learning Test Delayed Recall)	6	15
RAVLT (Rey Auditory Verbal Learning Recognition)	90	98
ROCF (Rey-Osterrieth Complex Figure Copy Immediate recall)	10	20
ROCF (Rey-Osterrieth Complex Figure Copy Delayed Recall)	12	24
FAB (Frontal Assessment Battery)	12	14
PVF (Phonological Verbal Fluency)	18	45
SVF (Semantic Verbal Fluency)	32	47
TMTA (Trail Making Test A)	50	49
TMTB (Trail Making Test B)	110	130
Verbal Span-Digit Forward	3.5	6
Verbal Span-Digit Back	4	5
Tas-20 (Toronto Alexithymia Scale-20)	40	55
BDI II (Beck Depression Scale)	12	15
AAT (Aachen Aphasia Test)	9	9
CBTT- Forward (Corsi block tapping task)	9	9
CBTT-Backward (Corsi block tapping task)	3	4
STEP Time (Time and Weight Estimation test)	14	21
STEP Weight (Time and Weight Estimation test)	13	24
STEP Total (Time and Weight Estimation test)	29	47
STAY Y-1 (State-Trait Anxiety Inventory)	45	60
STAY Y-2 (State-Trait Anxiety Inventory)	60	100

act not only locally with gastro-intestinal cells, but also directly with central nervous system (CNS) through metabolic and neuroendocrine pathways[2].

A recent study shows that IDO activity (and tryptophan metabolism), involved in the neuro-inflammation and participating in the onset of neurocognitive disorders, was significantly reduced by probiotic supplementation. In fact, IDO mRNA expression in the GALT and CSF neopterin levels decreased at the same time in cART treated HIV patients[8]. Moreover, according to results obtained in animal models, the manipulation of microbial taxa through probiotic supplementation may modulate the hippocampal regions controlling memory functions and attenuating age-related alterations in the hippocampus by increasing the brain-derived neurotrophic factor (BDNF) expression[39,40]. Based on these preliminary evidence probiotics supplementation seems to be an effective and safe approach to improve neurocognitive performance, and in particular memory functions, in HIV-1 positive patients.

As previous reported, the reduction in the levels of peripheral and CNS immune activation of was observed in cART treated patient after supplementation with specific multistrain probiotic formulation[8,9]. Given that neuroinflammation is considered a major contributor to the behavior and cognitive changes observed in HIV infection, probiotics could be a potentially useful tool to reduce the negative effects of immune activation also in the CNS, in association with cART therapy.

The improvement of cardio-metabolic assessment is usually well-known effect in subjects that have undergone regular physical activity: the increase of maximum rate of oxygen consumption (trough Rockport walk test) and an amelioration of lipid and glucose metabolism parameters are commonly reported. Less known are the potential benefits of regular physical exercise on immune-activation and neurocognitive performance. A comprehensive review of data available suggests that regular physical activity is a beneficial non-pharmacological intervention to reduce chronic inflammation, to improve the treatment of HIV residual disease and non-AIDS-defining illnesses in cART-treated HIV-infected individuals[11]. It remains to define the type and intensity of physical activity that is appropriate to diminish effectively the immune-activation, considering that it is well known that certain types of physical exercises may be pro-inflammatory stimuli[11].

CONCLUSIONS

Despite the limitations justified by the complexity of the matter, the available findings of studies indicate that neurocognitive functions are significantly improved after a long-term probiotic supplementation, supporting the hypothesis that modifications of the microbioma can provide specific neurological benefits in HIV-1 patients and reduce peripheral immune-activation. Also, physical activity seems to contribute to achieve the same goals, though other pathophysiological pathways. Our observations suggest that it is important to integrate these non-pharmacological tools into an overall approach based on a focus on the patient's lifestyle. Therefore, our findings seem to be encouraging, but large studies and clinical trials, carried out with specific methodological procedures, are needed to support the validity of current preliminary assumptions.

Conflict of Interests:
The Authors declare that they have no conflict of interests.

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