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IMPROVEMENT OF INTESTINAL PERMEABILITY WITH ALANYL-GLUTAMINE IN HIV PATIENTS: a randomized, double blinded, placebo-controlled clinical trial

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ABSTRACT – ***Context*** - Glutamine is the main source of energy of the enterocyte and diarrhea and weight loss are frequent in HIV infected patients. ***Objective*** - To determine the effect of alanyl-glutamine supplementation on intestinal permeability and absorption in these patients. ***Methods*** - Randomized double-blinded, placebo-controlled study using isonitrogenous doses of alanyl-glutamine (24 g/day) and placebo (glycine, 25 g/day) during 10 days. Before and after this nutritional supplementation lactulose and mannitol urinary excretion were determined by high performance liquid chromatography. ***Results*** - Forty six patients with HIV/AIDS, 36 of whom were male, with 37.28 ± 3 (mean ± standard error) years were enrolled. Twenty two and 24 subjects were treated with alanyl-glutamine and with glycine respectively. In nine patients among all in the study protocol that reported diarrhea in the 14 days preceding the beginning of the study, mannitol urinary excretion was significantly lower than patients who did not report this symptom [median (range): 10.51 (3.01–19.75) vs. 15.37 (3.93–46.73); $P = 0.0281$] and lactulose/mannitol ratio was significantly higher [median (range): 0.04 (0.00–2.89) vs. 0.02 (0.00–0.19); $P = 0.0317$]. There was also a significant increase in mannitol urinary excretion in the group treated with alanyl-glutamine [median (range): 14.38 (8.25–23.98) before vs 21.24 (6.27–32.99) after treatment; n = 14, $P = 0.0382$]. ***Conclusion*** - Our results suggest that the integrity and intestinal absorption are more intensely affected in patients with HIV/AIDS who recently have had diarrhea. Additionally, nutritional supplementation with alanyl-glutamine was associated with an improvement in intestinal absorption.

HEADINGS - Intestinal absorption. Glutamine. HIV. Placebos.

INTRODUCTION

HIV/AIDS figures among the top 10 diseases that affect global health and malnutrition represents the main risk factor that could affect human health[16]. Intense CD4$^+$ T lymphocytes depletion, enterocyte apoptosis, epithelial tight junction disruption and lymphoid cell infiltration occurs in the gastrointestinal tract of HIV/AIDS patients. Thus, HIV infection can be considered a disease of the gastrointestinal tract, given its involvement and importance since the very beginning of this disease[4].

Diarrhea is a major and frequent clinical manifestation of HIV infection in the tropics, often leading to malabsorption and significant impairment of nutritional status, quality of life and survival. In addition, little is known about the impact of this process on antiretroviral drugs absorption. Although the evidence demonstrates that malnutrition represents an independent risk factor for death[21, 23, 30], one can say that there is a shortage of therapeutic resources and specific formulas for nutritional support for patients with HIV/AIDS[20].

Among few existing options, glutamine stands out. There is now increasing evidence to recommend glutamine supplementation to patients with cancer, hematologic diseases and the critically ill. This amino acid, being a precursor of nucleotides synthesis, has great significance for rapidly dividing cells like occurs in the gut and in the immune system. So, it is

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reasonable to assume that its replacement is required when the endogenous production is not sufficient. The major site of glutamine release is the muscle and clinical conditions with significant muscle mass decrease could be an indication for exogenous replacement of this amino acid[33].

Untreated HIV infection is characterized by early loss of structural proteins of lean body mass and, more precisely, of body cell mass[30]. Therefore, we anticipated that glutamine supplementation should also be beneficial for HIV infected patients, especially those with diarrhea or weight loss. Alanyl-glutamine (AG) is replacing glutamine for clinical use due to its superior physicochemical solubility and more favorable thermal stability in acid medium[9]. This information enables us to propose this clinical trial to investigate the effect of AG supplementation on intestinal permeability and absorption in patients with HIV/AIDS in the era of highly active antiretroviral therapy (HAART).

METHODS

Study design and ethics

This is a prospective, double-blind randomized placebo-controlled trial. Study protocol and informed consent were approved by Ethical Committee of Hospital São José de Doenças Infecciosas, Fortaleza, CE, Brazil.

Selection and enrollment of participants

Fifty one HIV positive adults outpatients treated at Hospital São José de Doenças Infecciosas, in Fortaleza, CE, northeast of Brazil, were invited to participate in the trial as they attended for their routine medical appointment. Those presenting one or more of the following conditions were excluded: severe dehydration, serious systemic diseases such tuberculosis, histoplasmosis, malignancies, active pneumonia, meningitis, sepsis, receiving parenteral nutrition, renal, cardiac or hepatic impairment, pregnancy, inability to receive food and/or oral medications and unable to give informed consent. Three patients did not agree to participate for fear that the home visit to receive the medication could identify their status as HIV positive and two others were afraid to participate in a clinical research. So, 46 subjects were enrolled in the trial.

Intervention regimen and study duration

Patients were allocated into two blinded groups by restricted randomization by use of random permuted blocks of adequate lengths and follow the protocol summarized in Figure 1. AG group was randomized to receive 10 days of an oral solution of 50 mL of orange juice enriched with 24 g of alanyl-glutamine (Rexim S.A., Degussa Group, Courbevoie, France). Glycine (GLY) group was randomized to receive as placebo, the same volume of an oral solution of orange juice enriched with 25 g of GLY (Spectrum Chemical, Gardena, CA) for 10 days, with the same nitrogen concentration, appearance and taste of the solution enriched with AG. Treatment intake were directly observed and registered by home visitors.

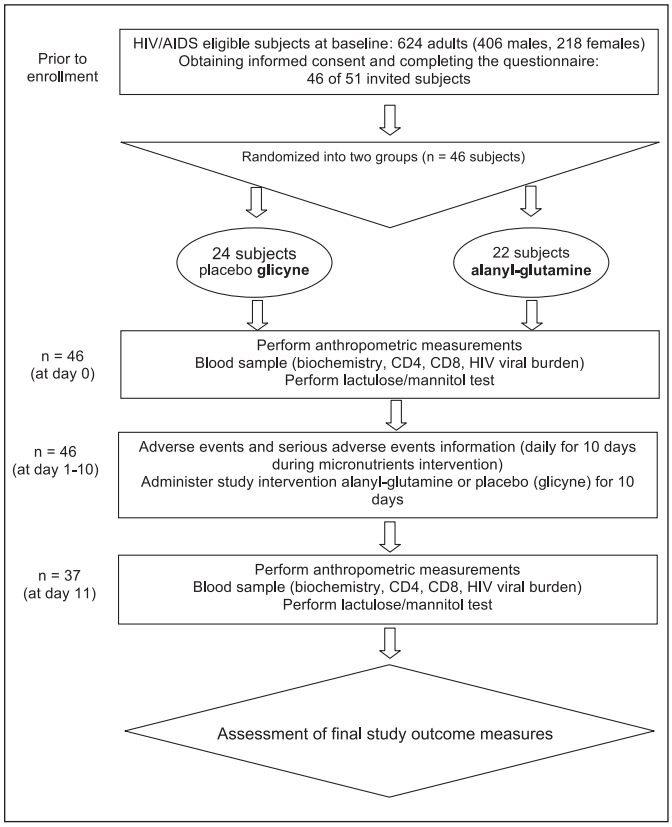

FIGURE 1. Flow diagram for study protocol

After enrollment, anthropometric measures, complete blood count, blood biochemistry, CD4 and HIV viral load were performed and patients were scheduled to perform the intestinal permeability test and to begin the proposed treatment. Those procedures were performed again 1 day after the end of treatment with AG or GLY (day 11).

Intestinal permeability test

After overnight fast and emptying the bladder, each enrolled subject ingested a solution containing 5 g of lactulose (Duphar Laboratories, Southampton, UK) and 1 g of mannitol (Henrifarma Produtos Químicos e Farmacêuticos Ltda, São Paulo, Brazil) dissolved in 20 mL of water. Urine was collected during the next 5 hours after administration of the double sugar solution. Then, the total volume was recorded, and an aliquot of 5 mL preserved with chlorhexidine (0.236 mg/mL of urine; Sigma Chemical, St Louis, MO, USA) was storage at -20°C for sugar analysis. The lactulose and mannitol concentrations were measured by HPLC (high performance liquid chromatography) as previously described[1].

Adverse events and serious adverse events monitoring

Adverse events (AE) and serious adverse events (SAE) were monitored daily by study staff. An AE was defined as any untoward medical occurrence that may arise during

administration of the AG or placebo GLY and that may or may not have a causal relationship with the study agent. A SAE was defined as any adverse experience occurring that resulted in any of the following outcomes: death, life threat, requirement for inpatient hospitalization, persistent or significant disability or incapacity, or an important medical event. All AE and SAE were recorded, reviewed by qualified staff, and reported to Ethics Committee.

Statistics

The lactulose/mannitol ratio was selected as the primary outcome variable to calculate the sample size because it was objective data and because it reflected overall intestinal barrier disruption. On the basis of data from preliminary studies (lactulose/mannitol ratio = 0.0146 ± 0.0036) we calculated that a sample size of 21 patients in both the control and the experimental groups would give 90% power to detect a reduction of 30% or more in lactulose/mannitol ratios at a significance level of $P = 0.05$[1].

Statistical analysis was performed using de Statistical Package for Social Sciences (version 11.5, SPSS Inc., Chicago, IL, USA). Lactulose and mannitol results were log transformed before submission for statistical analysis. To assess the homogeneity of the study groups Student *t* test and c^2 and Fisher exact test were used. To evaluate changes after nutritional supplementation paired Student *t* test was used. Values were considered statistically significant when $P \leq 0.05$.

RESULTS

According to the Epidemiological Surveillance Unit of Hospital São José de Doenças Infecciosas, 624 HIV infected adults (406 male; 218 female) were under medical attention at this hospital at the baseline. Of these 624 patients, 51 were invited while attended at the hospital for routine medical consultation and 46 agreed to participate in the clinical study. After blinded randomization, 22 were allocated to receive AG and 24 to receive GLY (placebo). In AG group five patients discontinued participation: two by personal decision; one due to diarrhea and fever; one due to dizziness, nausea and vomiting; and another one was hospitalized as a result of head injury in motorcycle accident. In this group, therefore, 17 patients completed the clinical study as proposed initially. In GLY group, four patients discontinued the study for the following reasons: one due to nausea and vomiting; one due to nausea, headache and dizziness; one due to nausea, sweating, asthenia and blurred vision; and one died with suspected histoplasmosis. Therefore in this group 20 patients completed the intervention. Figure 1 provides a flow diagram of the population selected for the study.

Baseline demographic and clinical characteristics of enrolled patients by intervention groups, age, gender, clinical classification of HIV infection according to the CDC[7], CD4, HIV viral load, antiretroviral usage, body mass index (BMI) and report of diarrhea in the last 14 days are summarized

TABLE 1. Patients characteristics at baseline according to age, sex, clinical classification, CD4, HIV viral load, antiretroviral therapy, weight, body mass index and diarrhea in the last 14 days

Parameters	All	Study groups		*P*
	n = 46	Placebo GLY [a] n = 24	AG [b] n = 22	
Age in years (Mean ± SE)	37.28 ± 3.00	40.13 ± 1.89	34.18 ± 1.66	0.024*
Gender				
Male, n (%)	36 (78.3)	17 (70.8)	19 (86.4)	NS
Female, n (%)	10 (21.7)	7 (29.2)	3 (13.6)	NS
Clinical classification [c]				
A, n (%)	15 (32.6)	7 (29.2)	8 (36.4)	NS
B, n (%)	3 (6.5)	1 (4.2)	2 (9.1)	NS
C, n (%)	28 (60.9)	16 (66.6)	12 (54.5)	NS
CD4/mm^3, n (Mean ± SE)	44 (384.5 ± 16.47)	24 (409.5 ± 64.9)	20 (354.4 ± 46.0)	NS
<200, n (%)	12 (27.3)	7 (29.2)	5 (25.0))	NS
≥200, n (%)	32 (72.7)	17 (70.8)	15 (75.0)	NS
VL (log_{10}) , n (Mean ± SE)	37 (4.08 ± 1.05)	22 (4.08 ± 1.11)	15 (4.05 ± 0.99)	NS
HAART				
Yes, n (%)	38 (82.6)	22 (91.7)	16 (72.7)	NS
No, n (%)	8 (17.4)	2 (8.3)	6 (27.3)	NS
Weight, n (Median ± SE)	46 (60.9 ± 3.3)	24 (60.7 ± 2.4)	22 (62.2 ± 2.1)	NS
BMI , n (Median ± SE)	46 (22.9 ± 1.9)	24 (22.3 ± 0.7)	22 (22.4 ± 0.8)	NS
Diarrhea (last 14 days)				
Yes, n (%)	9 (19.6)	6 (25.0)	3 (13.6)	NS
No, n (%)	37 (80.4)	18 (75.0)	19 (86.4)	NS

[a] GLY = glycine (placebo) – GLY (25 g/day) was given with orange juice for 10 days, beginning on the 1st day of the study protocol
[b] AG = alanyl-glutamine - AG (24 g/day) was given with orange juice for 10 days, beginning on the 1st day of the study protocol
[c] CDC (1993)[7]
SE = standard error; NS = not significant; VL = HIV viral load; HAART = highly active antiretroviral therapy; BMI = body mass index
* $P = 0.024$ comparing the mean age between AG group and the GLY (placebo) group using the Student *t* test

in Table 1. There were no significant statistical differences of these parameters by treatment groups, except for age (40.13 ± 1.89 years in GLY group vs 34.18 ± 1.66 years in AG group; $P = 0.024$).

As shown in Table 2, at baseline there were no statistically significant differences in the intestinal permeability test between AG and GLY groups, nor according to use of antiretroviral therapy, CD4 count and the BMI. Among nine patients who had reported diarrhea in the last 14 days preceding the beginning of the study the urinary excretion of mannitol was significantly lower [median (range): 10.51 (3.01–19.75) vs 15.37 (3.93–46.73); $P = 0.0281$] and lactulose/mannitol ratio was significantly higher [median (range): 0.04 (0.00–2.89) vs 0.02 (0.00–0.19); $P = 0.0317$] when compared with patients who did not report diarrhea in this same period.

There were no significant differences between AG and GLY groups in hemoglobin level, hematocrit, leukocyte and platelet count, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea and creatinine before and after the intervention proposed. There were also no significant differences in weight, BMI, HIV viral load, CD4, urinary excretion of lactulose and urinary lactulose/mannitol ratio between treatment groups (Table 3). Inversely, there was a

TABLE 2. Intestinal permeability test parameters at baseline according to treatment groups, diarrhea occurrence in the last 14 days, use of HAART, CD4 and body mass index

Intestinal permeability test parameters median (range)	Treatment group or clinical status		*P*
	Placebo GLY[a] (n = 24)	**AG [b] (n = 22)**	
% of lactulose excretion	0.32 (0.00–8.71)	0.30 (0.00–3.82)	NS
% of mannitol excretion	16.10 (3.01–46.70)	13.70 (5.30–31.60)	NS
Lactulose/mannitol ratio	0.02 (0.00–2.89)	0.02 (0.00–0.19)	NS
	Diarrhea reported in the last 14 days (n = 9)	**No diarrhea reported in the last 14 days (n = 36)**	
% of lactulose excretion	0.86 (0.00–8.71)	0.29 (0.00–3.82)	0.0318*
% of mannitol excretion	11.70 (3.01–22.20)	15.35 (3.90–46.70)	NS
lactulose/mannitol ratio	0.05 (0.00–2.89)	0.02 (0.00–0.19)	0.0261*
	Without HAART (n = 8)	**HAART use (n = 37)**	
% of lactulose excretion	0.33 (0.10–1.01)	0.29 (0.00–8.71)	NS
% of mannitol excretion	17.05 (5.30–43.60)	14.00 (3.01–46.70)	NS
Lactulose/mannitol ratio	0.03 (0.00–0.19)	0.02 (0.00–2.89)	NS
	CD4 <200/mm^3 (n = 12)	**CD4 ≥200/mm^3 (n = 31)**	
% of lactulose excretion	0.32 (0.00–1.38)	0.32 (0.00–8.71)	NS
% of mannitol excretion	13.60 (6.40–46.70)	14.50 (3.01–43.60)	NS
Lactulose/mannitol ratio	0.02 (0.00–0.22)	0.02 (0.00–2.89)	NS
	BMI <18.5 (n = 4)	**BMI ≥18.5 (n = 42)**	
% of lactulose excretion	0.67 (0.42–1.16)	0.29 (0.00–8.71)	NS
% of mannitol excretion	12.50 (5.40–31.60)	14.50 (3.01–46.70)	NS
lactulose/mannitol ratio	0.07 (0.01–0.13)	0.02 (0.00–2.89)	NS

[a] GLY = Glycine (placebo) – GLY (25 g/day) was given with orange juice for 10 days, beginning on the 1st day of the study protocol

[b] AG = alanyl-glutamine - AG (24 g/day) was given with orange juice for 10 days, beginning on the 1st day of the study protocol

HAART = highly active antiretroviral therapy; NS = not significant; BMI = body mass index

* $P = 0.0318$ and $P = 0.0261$ comparing groups that reported and did not report diarrhea in the last 14 days at baseline, using the Student *t* test, for urinary excretion of lactulose (%) and for lactulose/mannitol ratio respectively

significant increase in the percentage of mannitol urinary excretion in the AG group [median (range)] after treatment: [14.38 (8.25–23.98) before treatment vs 21.24 (6.27–32.99) after treatment; n = 14, $P = 0.0382$], and no difference in GLY (placebo) group as shown in Table 3.

There were two SAE, one in each treatment group. In the AG group one patient was hospitalized as a result of head injury in a motorcycle accident. In the GLY group one patient died with suspected disseminated histoplasmosis. This patient was asymptomatic during his study treatment and when he presented at the 11th day to perform his intestinal absorption test he suddenly began to develop respiratory distress and was hospitalized and died after 4 days. AE do not differed significantly between treatment groups (Table 4).

TABLE 3. Changes in weight, BMI, CD4 count, viral load and intestinal permeability test after treatment with glycine (placebo) or alanyl-glutamine

Parameters	Treatment group			
	Placebo glycyne [a]		Alanyl-glutamine [b]	
Weight (kg)	**n = 20**	***P***	**n = 17**	***P***
Before (mean ± SE)	60.64 ± 2.59	NS	63.08 ± 2.04	NS
After (mean ± SE)	62.66 ± 2.82		63.82 ± 1.98	
BMI	**n = 20**		**n = 17**	
Before (mean ± SE)	23.75 ± 0.82	NS	22.62 ± 0.87	NS
After (mean ± SE)	23.73 ± 0.99		22.87 ± 0.86	
CD4 (/mm^3)	**n = 19**		**n = 17**	
Before (mean ± SE)	421.26 ± 75.84	NS	384.88 ± 48.48	NS
After (mean ± SE)	278.45 ± 63.88		412.00 ± 54.97	
HIV viral load (log_{10})	**n = 19**		**n = 9**	
Before (mean ± SE)	4.04 ± 0.56	NS	4.07 ± 0.99	NS
After (mean ± SE)	3.86 ± 0.63		3.71 ± 0.72	
% of lactulose excretion	**n = 19**		**n = 14**	
Before, median (range)	0.28 (0.00–1.15)	NS	0.21 (0.00–3.82)	NS
After, median (range)	0.29 (0.00–3.95)		0.26 (0.00–1.17)	
% of mannitol excretion	**n = 19**		**n = 14**	
Before, median (range)	16.79 (3.93–46.73)	NS	14.38 (8.25–23.98)	0.0382*
After, median (range)	17.47 (0.00–47.51)		21.24 (6.27–32.99)	
Lactulose/mannitol ratio	**n = 19**		**n = 14**	
Before, median (range)	0.02 (0.00–0.12)	NS	0.02 (0.00–0.19)	NS
After, median (range)	0.02 (0.00–0.24)		0.01 (0.00–0.41)	

[a] GLY = Glycine (placebo) – GLY (25 g/day) was given with orange juice for 10 days, beginning on the 1st day of the study protocol
[b] AG = alanyl-glutamine - AG (24 g/day) was given with orange juice for 10 days, beginning on the 1st day of the study protocol
BMI = body mass index; SE = standard error; NS = not significant
* $P = 0.0382$ comparing the percentage of mannitol urinary excretion before and after treatment in AG group, using paired Student *t* test

TABLE 4. Adverse events and serious adverse events according to treatment groups with alanyl-glutamine or glycine (placebo)

Parameters	Treatment groups					
	Placebo glycine [a] (n = 24)		Alanyl-glutamine [b] (n = 22)		Total (n = 46)	
	n	%	n	%	n	%
Adverse event [c]						
Dizziness	4	14.81	4	16.00	8	15.38
Nausea	4	14.81	4	16.00	8	15.38
Asthenia	2	7.40	2	8.00	4	7.69
Vomit	2	7.40	2	8.00	3	5.76
Facial paresthesia	2	7.40	0	0.00	2	3.84
Sweating	1	3.70	1	4.00	2	3.84
Cold extremities	1	3.70	1	4.00	2	3.84
Blurred vision	1	3.70	0	0.00	1	1.92
Headache	1	3.70	0	0.00	1	1.92
Constipation	1	3.70	0	0.00	1	1.92
Colic	1	3.70	0	0.00	1	1.92
Burning in thorax	1	3.70	0	0.00	1	1.92
Diarrhea	0	0.00	1	4.00	1	1.92
Fever	0	0.00	1	4.00	1	1.92
Paresthesia of members	0	0.00	1	4.00	1	1.92
Dyspnea	0	0.00	1	4.00	1	192
Serious adverse event [d]						
Death [e]	1	3.70	0	0.00	1	1.92
Head injury [f]	0	0.00	1	4.00	1	1.92

[a] GLY = Glycine (placebo) – GLY (25 g/day) was given with orange juice for 10 days, beginning on the 1st day of the study protocol
[b] AG = alanyl-glutamine - AG (24 g/day) was given with orange juice for 10 days, beginning on the 1st day of the study protocol
[c, d] $P > 0.05$ comparing treatment with versus GLY (Chi-square or Fisher's exact test)
[e] Clinical feature of disseminated histoplasmosis
[f] Motorcycle accident

DISCUSSION

Our study was proposed to evaluate the ability of glutamine on improving intestinal function of HIV infected patients. For this purpose we have used AG, a dipeptide which physicochemical stability has been shown to be superior to the use of glutamine. In addition, glutamine hydrolysis tends to form glutamate, which is potentially toxic to the central nervous system. This problem does not happen with AG[10].

We observed that there was a significant difference in age between treatment groups, which was lower in AG group (34.18 versus 40.13 years; $P = 0.024$). This statistic, however, seems devoid of importance to influence the outcome of the study (Table 1).

Comparing intestinal permeability tests results in our study with the extremes of normality previously reported in healthy individuals in Brazil[32], 56.5%[19] of our patients had greater urinary excretion of lactulose [0.32 (0.00-8.72) vs 0.07 (0.05–0.28)], 60.9%[2] had lesser urinary excretion of mannitol [14.56 (3.02-124.11) vs 21 (18.30–28.00)], and 67.4%[25] had a greater lactulose/mannitol ratio [0.02 (0.00–2.89) vs 0.003 (0.002–0.013)]. Soares et al.[28] had previously reported that in HIV/AIDS patients, with or without diarrhea or weight loss, D-xilose absorption test was abnormal when compared with healthy control subjects. In agreement with those findings, our data showed that most patients with HIV/AIDS still presents intestinal impaired absorption and altered permeability, even in the era of HAART.

Diarrhea was reported as having occurred less than 14 days before the baseline by nine (19.6%) patients (Table 1). As shown in Table 2, urinary mannitol excretion was significantly lower [median (range): 10.51 (3.01–19.75) vs 15.37 (3.93–46.73); $P = 0.0281$] and lactulose/mannitol ratio was significantly higher [median (range): 0.04 (0.00–2.89) vs 0.02 (0.00–0.19); $P = 0.0317$] in this group than among patients who did not report diarrhea. This result is similar to the data previously reported by Lima et al.[15] published before the advent of HAART. The advantage of an intestinal absorption test that uses two sugars with distinct molecular sizes is to make possible to evaluate at the same time intestinal lesion (increased urinary excretion of lactulose) and a change in intestinal absorption surface (reduced excretion of mannitol), as reported by Sorensen et al.[29]. Our data shows the impact of diarrhea in intestinal absorption in patients with HIV/AIDS, mainly suggesting the reduction of intestinal absorption surface.

Potentially less favorable clinical conditions such as CD4 lower than 200/mm^3, BMI lower than 18.5 and being in use of antiretroviral therapy (Table 2) did not represent conditions that significantly altered intestinal permeability tests at baseline. Perhaps this could be explained because the majority of patients were already on HAART or it might have been necessary the inclusion of a greater number of patients to find differences between those groups.

We choose GLY as placebo. GLY did not interfere in the treatment of diarrhea when added to an oral rehydration solution in a previous study[31]. Additionally, the use of GLY has made possible providing the required equal amount of nitrogen for the two treatment groups.

There are increasing evidences that glutamine may act as a vital signaling molecule to the cells in the event of illness or trauma, able to play a regulatory role of several genes associated with metabolism, signal transduction, cell repair and defense and also to signalize activation of intracellular pathways[8]. It is therefore possible that the release of glutamine by muscle or other tissues acts as a "stress signaling" to the body to the activation of vital genes for cell protection and immune regulation[34].

During catabolism states a large amount of amino acids, including glutamine, is released from the muscle tissue. Therefore, it is expected that, as in critical patients that has evidenced low serum levels of glutamine, the same occurs in patients living with HIV/AIDS[22, 24]. So, it is reasonable to assume that dietary supplementation with glutamine may have a positive impact on the nutritional status of these patients. As can be seen in Table 3 there were no significant changes in anthropometric parameters in the group that received AG, neither in the group that received GLY. This could be explained because we did not make subsequent anthropometric evaluations and by the short period of treatment used in our protocol. Shabert et al.[26], using a different study design from ours and in which treatment with glutamine (40 g/day) for HIV infected patients continued for 3 months, observed 2.2 kg of median weight gain, 1 kg of body cell mass gain and an increase of intracellular water.

One of the striking features of the immune system is the high rate of consumption of glutamine by its cells in culture media[18]. Hack et al.[13], for instance, described a strong association between the fall in serum levels of glutamine and the decrease of T CD4$^+$ lymphocytes ($r = 0.67$, $P<0.001$) of a group of individuals after 8 weeks of a program of physical exercises. On his turn, Jing-Xiang et al.[14] observed a significant increase in the count of T CD4$^+$ lymphocytes and also of CD4/CD8 ratio on the 4th post-operative day of a group of patients with colorectal cancer who received parenteral nutrition enriched with AG. In AG group of our trial there was an increase in the absolute count and in the percentage of T CD4$^+$ lymphocytes. Inversely, in GLY group we observed a reduction in these parameters after 10 days of treatment (Table 3). However, those differences were not statistically significant. In the light of recent concepts about the importance of maintaining the integrity of the mucosal barrier to inhibit microbial translocation and thus prevent the systemic immune activation and consequent depletion of T CD4$^+$ cells[3], our result of increase in the absolute count and percentage of T CD4$^+$ lymphocytes in the AG group is particularly exciting about a possible role of glutamine in preservation and repair of intestinal mucosa.

No significant changes in urinary lactulose excretion and on lactulose/mannitol ratio were observed in either treatment group (Table 3). However, there was a significant increase in urinary excretion of mannitol in AG group [median (range): 14.38 (8.25–23.98) before vs 21.24 (6.27–32.99) after treatment; $P = 0.0382$] as shown in Table 3. These results may

suggest that the initial effect of AG in HIV infected patients seems to be the recovery of intestinal absorptive area and, subsequently, the repair of the intestinal barrier lesion. In agreement with our findings, Noyer et al.[19] have conducted a study in which a group of patients received glutamine (8 g/day) during 28 days and also presented an increase in urinary excretion of mannitol, although not significant. In another clinical trial, the group of patients randomized to receive higher doses of AG and glutamine for 1 week also showed a tendency to have an increase in the urinary excretion of mannitol, although not significant. Also in this study there has been no significant change in the urinary excretion of lactulose[5]. Taken together, these results may suggest that both the dose of AG or glutamine used as the treatment duration are important in order to achieve an effect on the absorption and on rupture repair of intestinal barrier in patients with HIV/AIDS. Our result is also consistent with the observations of Batman et al.[2], who suggest that the cell hyperplasia of crypts could represent the primary lesion of HIV enteropathy, determining the migration of immature enterocytes into the villi and, thus, leading to a reduction of the intestinal absorption area by mobilizing the villi from the crypt-villi junction toward the lumen.

Considering the relative scarcity of studies dealing with glutamine supplementation safety[32] we established a control system for AE and SAE in our clinical trial. The most common AE were dizziness and nausea, with no significant differences between the treatment and placebo (glycine) groups (Table 4). All AE resolved without sequel and neither one of the two SAE was definitively linked with either of the two treatments.

Recent studies of HIV and simian immunodeficiency virus (SIV) infections have shown a decrease in the expression of genes associated with repair and regeneration of the intestinal mucosa, including several factors of cell growth and proliferation[11, 12, 25]. Those studies suggest that the processes of inflammation and regeneration of the mucosa may be object of modulation through the development of new drugs able to stimulate the restoration of the intestinal mucosal immune system and the repair of the GALT. Curi et al.[8] suggested that glutamine has a regulatory role of several genes related to metabolism, to signaling transduction, to cell defense and to repairing and activating of intracellular pathways signaling. Thus, pharmacogenomics studies with this amino acid seem to be more than ever on the agenda.

In conclusion, in agreement with others[6, 17, 27], we observed that diarrhea remains a clinical problem among patients with HIV/AIDS, even among those taking HAART, and that diarrhea has a significant impact on intestinal permeability. Additionally, AG was safe and able to produce an effect of improvement in intestinal absorption area in HIV infected patients, as suggested by the finding of a significant increase in the percentage of urinary excretion of mannitol, and thus deserves further study in ameliorating the diarrhea and malabsorption that remains a problem for patients with HIV/AIDS.

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Leite RD, Lima NL, Leite CAC, Farhat CK, Guerrant RL, Lima AAM. Melhora da permeabilidade intestinal com o uso de alanil-glutamina em pacientes infectados pelo HIV: ensaio clínico duplo-cego, randomizado, controlado por placebo. Arq Gastroenterol. 2013;50(1):56-63.

RESUMO - ***Contexto*** - A glutamina é a principal fonte de energia do enterócito e diarreia e perda de peso são frequentes em pacientes infectados pelo HIV. ***Objetivo*** - Determinar o efeito da alanil-glutamina sobre a permeabilidade e a absorção intestinais nesses pacientes. ***Métodos*** - Estudo duplo-cego, randomizado, controlado por placebo, utilizando doses isonitrogênicas de alanil-glutamina (24 g/dia) e de placebo (glicina, 25 g/dia) durante 10 dias. Antes e depois dessa suplementação nutricional a excreção urinária de lactulose e manitol foi determinada por cromatografia líquida de alta performance. ***Resultados*** - Quarenta e seis pacientes com HIV/AIDS, sendo 36 do sexo masculino, com 37,28 ± 3 anos (média ± erro padrão) foram incluídos. Vinte e dois e 24 indivíduos foram tratados com alanil-glutamina e com glicina, respectivamente. Nos nove pacientes que relataram ter apresentado diarreia nos 14 dias anteriores ao início do estudo, a excreção urinária de manitol foi significativamente menor do que nos pacientes que não referiram essa queixa [mediana (intervalo): 10,51 (3,01-19,75) vs 15,37 (3,93-46,73), P = 0,0281] e a razão lactulose/manitol foi significativamente mais elevada [mediana (intervalo): 0,04 (0,00-2,89) vs 0,02 (0,00-0,19), P = 0,0317]. Constatou-se também aumento significativo na excreção urinária de manitol no grupo tratado com alanil-glutamina [mediana (intervalo): 14,38 (8,25-23,98), antes vs 21,24 (6,27-32,99) após o tratamento, n = 14, P = 0,0382]. ***Conclusão*** - Os resultados do presente estudo sugerem que a integridade e a absorção intestinais são mais intensamente afetadas em pacientes com HIV/AIDS que tiveram diarreia recentemente. Adicionalmente, a suplementação nutricional com alanil-glutamina associou-se à melhoria na absorção intestinal.

DESCRITORES – Absorção intestinal. Glutamina. HIV. Placebo.

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