

In Vitro* Effect of Folic Acid and Cobalamin (Vitamin B12) on Adhesion and Growth of *Giardia lamblia

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Abstract

Giardia lamblia is one of the most common intestinal protozoan parasites infecting human in the world. The goal of this study was searching for *in-vitro* effect of folic acid and cobalamin on adhesion and growth of *G. lamblia* as two important mechanisms in the pathogenesis in TYI-S-33 medium. *G. lamblia* trophozoites were obtained by *in-vitro* excystation procedure. Three groups of *Giardia* trophozoites were analyzed: control group, *G. lamblia* was cultured in TYI-S-33 without any vitamin, 2nd group with 0.1 µg/ml vitamin B12 or folic acid, and 3rd group with 0.5 µg/ml of vitamin B12 or folic acid. All culture media tubes incubated at 37 °C. After 2 h of incubation, the adherence into borosilicate culture tubes, and after 24 h the growth of trophozoites were measured. The results showed that in vitamin B12 groups, the growth was increased significantly ($P \leq 0.05$) but the adherence decreased significantly ($P \leq 0.05$). Folic acid inhibited the growth rate significantly ($P \leq 0.05$), but it increased adherence in axenic culture significantly ($P \leq 0.05$). The results showed that vitamin B12 and folic acid altogether might reduce pathogenesis of *G. lamblia* by reducing adherence and growth, respectively.

Keywords: *Giardia lamblia*, Cobalamin (Vitamin B12), Folic Acid

Introduction

The micro aerophilic flagellated protozoan parasite, *Giardia lamblia* is the most common intestinal parasites infecting human, particularly children in the world (1). *Giardia* is one of the most important factors in persistent diarrhea in children. Persistent diarrhea seriously affects nutritional status, growth, and intellectual function (2). In pregnant women statistical differences of common symptoms such as nausea, between pregnant women infected with *Giardia* and those without any parasitic infection were significant ($P < 0.05$) (3).

Symptomatic infection is characterized by dehydration, bloating, fever, cramps, malaise, weight loss, diarrhea, epigastric pain,

nausea, and often aggravated by poor levels of nutrition and hygiene (4- 6).

G. lamblia mostly colonizes in duodenum and jejunum (7). Damage of intestinal epithelium by adherent trophozoites of *G. lamblia* has been observed as one important mechanism in the pathogenesis of the infection (8). *In-vivo* attachment is important because it permits *Giardia* spp. to remain in a physiologically favorable environment and avoid elimination by peristaltic movement of the intestine (9). This can be led to endothelial dysfunction and malabsorption of essential nutrients. The structural modifications produced by *G. lamblia* trophozoites of epithelial cells are the result of close attachment of a contractile region of the ventral disc (8).

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Giardiasis causes deficiency of vitamin B₁₂ and can also inflame the bowel and interfere in folate absorption (10-11).

B₁₂ as a co-enzyme is needed for enzymes to do their function of changing one molecule into another. One part of its structure is known as the corrin nucleus. In B₁₂, the corrin holds an atom of cobalt. The corrin plus other atoms makes up the part of B₁₂ known as cobalamin. Depending on the attachment, cobalamin becomes cyanocobalamin, hydroxocobalamin, aquocobalamin, nitritocobalamin, methylcobalamin, or adeno-sylcobalamin (also called 5'-deoxyadenosylcobalamin) (12).

Only 2 cobalamins are active as co-enzymes in the human body: adenosylcobalamin and methylcobalamin. However, the body has the ability to convert most other cobalamins into one of these active forms. Cyanocobalamin is the form most often found in vitamin tablets because it is one of the most stable forms of cobalamin and the body readily converts it into one of the useable co-enzymes (13).

Folic acid, also known as folate, is a B-vitamin (B₉) that can be found in some enriched foods and vitamin pills (14). Vitamin folate comes into play in B₁₂ deficiency and people with a folate deficiency can have the problem known as megaloblastic anemia or macrocytic anemia. Vitamin B₁₂ helps to transport and store folate in the cells (15). Folic acid is actually a very important vitamin and growing cells need folic acid. Folate must be digested by pancreatic juice in the duodenum, where *G.lamblia* usually colonizes. B₁₂ and B₉ are dissolved in water (11). Deficiency of folic acid is not necessarily due to poor diet. Intestinal malabsorption causes low folic acid levels. Deficiency of folic acid, even if temporary found to weaken the immune system for about 3 months especially in patients with immunodeficiency. It is a good

recommended to check folic acid levels in anyone who suffers from persistent intestinal symptoms, particularly of excessive gas and bloating (11). The most important function of folic acid is in the manufacture of nucleic acid, essentially for growth and repair in every cell in the body (11).

Plasma folate concentrations were measured by several studies simultaneously in the assay for vitamin B₁₂ and malabsorption and deficiency of both vitamins. For example, in 29 Swedish children age 0.7-13.5 yr (mean 3.3 yr) with chronic giardiasis, subnormal fractional absorptions of folate and vitamin B₁₂ were present in one sixth and one third of the children, respectively (16). In Peruvian adults with megaloblastic anemia associated with chronic diarrhea and small bowel bacterial overgrowth, 64% had both low serum vitamin B₁₂ and folate, 20% had low serum vitamin B₁₂, and 16% had low serum folate alone (17).

The aim of this study was *in-vitro* evaluation of vitamin B₁₂ and folic acid effect on growth and adherence of *G.lamblia* trophozoites.

Materials and Methods

G. lamblia cyst isolation

G. lamblia cysts were isolated from fresh faeces of patients with giardiasis (3 isolates). *G. lamblia* cysts were purified and concentrated from faeces combining the sucrose flotation method with a simplified sucrose gradient method (18). The cysts, after being washed twice in distilled water, were resuspended in distilled water and stored at 4 °C for maximum of 3 days prior to use.

Excystation and axenization

The excystation procedure was a modification of the Bingham & Meyer technique (19). Briefly, in the excystation procedure, 1 volume of clean cysts was added to

9 volumes of HCl in pH= 2 for 30 min, and incubated at 37 °C. Then the cysts were inoculated into the culture tubes (Screw-capped borosilicate culture tubes) containing 13 ml of modified TYI-S-33 medium.

Vitamins preparation

Vitamin B12 was dissolved in distilled water in pH= 7 and folic acid dissolved in distilled water in pH= 3-4. We used two concentrations of each vitamin, 0.1 and 0.5 µg/ml.

Trophozoites were harvested by chilling the tubes in ice water for 5 min. Trophozoites were counted by heamocytometer, and 5×10^5 trophozoites were cultured, then vitamin B12 and folic acid were added.

Three groups of *Giardia* trophozoites were analysed: 1- control group, *G. lamblia* cultured in TYI-S-33 without any vitamin, 2nd group with 0.1 µg/ml vitamin B12 or folic acid, and 3rd group with 0.5 µg/ml of vitamin B12 or folic acid .

Adherence inhibition assay

For adherence inhibition assay, 5×10^5 trophozoites were cultured, then 0.1 and 0.5 µg/ml amounts from each vitamin pipetted into borosilicate culture tubes and incubated at 37 °C, for 2 h. The effluents were collected in disposable test tubes and for enumeration of adhered trophozoites to borosilicate culture tubes, the tubes chilled in ice for 5 min then trophozoite concentrations calculated with a heamocytometer. Experiments were performed in triplicate and the results calculated as follows (4):

$$\text{Percentage adherence} = 100 - \left(\frac{C_e}{C_o} \times 100 \right)$$

C_e was the concentration of *Giardia* in the effluent, and C_o was the concentration in the original suspension.

Growth inhibition assay

For this experiment, 24 h cultures of *G. lamblia* were harvested as previously described, which involved 0.1 and 0.5 µg/ml of each vitamin. Control culture was in the same condition except no vitamins.

These cultures were kept at 37 °C for 24 h, after which they were chilled and the trophozoites concentrations determined by the help of haemocytometer.

Experiments were performed in triplicate, and the results calculated as follows (4):

Percentage growth

$$\text{Inhibition} = 100 - \frac{\text{Giardia concentration in vitamin-treated}}{\text{Giardia concentration in untreated culture}} \times 100$$

Results

Effect of Cobalamin (vitamin B₁₂)

The effect of vitamin B₁₂ on growth rate and percentage of adherence are summarized in Table 1. As the result showed vitamin B₁₂ increased the growth rate but decreased the adherence and the differences were significant when compared with control group (vitamin B₁₂ concentration= 0) ($P \leq 0.05$).

Table 1: The *in-vitro* effect of vitamin B12 on growth rate and adherence of *G.lamblia*

B12 concentration (µg/ml)	Growth rate	% of adherence
0 (control group)	120	50
0.1	* 240	25*
0.5	* 530	17*

*statistical significant differences ($P < 0.05$)

***Effect of folic acid (vitamin B₉)**

In the other set of experiments, we investigated the in vitro effects of folic acid (vitamin B₉) on growth and adherence of *G. lamblia*. The results showed that folic acid significantly inhibited growth rate in axenic culture, but increased adherence and the differences were significant when compared with control group (vitamin B₉ concentration= 0) ($P \leq 0.05$). The results are summarized in Table 2.

Table 2: The effect of folic acid on *in-vitro* growth rate and adherence of *G. lamblia*

B9 concentration (µg/ml)	Growth rate	% of Adherence
0 (control group)	120	50
0.1	78*	67*
0.5	32*	82*

*statistical significant differences ($P < 0.05$)

Discussion

G. lamblia, the flagellated protozoa is causative agent of giardiasis. The common symptoms of giardiasis are diarrhea, fever, abdominal pain, weight loss, dehydration, and nausea. The most important pathophysiological effect of this protozoan is adherence (8). A dense coating of flagellation on the intestinal epithelium interferes with the absorption of fats and other nutrients, which probably triggers the onset of disease (20). Combination of parasitic factors and host responses seem to be adherent trophozoites of *G. lamblia* and damage of the intestinal epithelium by adherent trophozoites of *G. lamblia* has been proposed as one important mechanism in the pathogenesis of the infection (21-22). Adherence disorders the absorption and epithelial cells in giardiasis and reduces plasma level of mineral,

folic acid and vitamin B₁₂ (23). The vitamin folate comes into play in B₁₂ deficiency. Folate is needed to produce DNA. In creating methylcobalamin, B₁₂ takes a methyl group from one form of folate. Therefore, it produces a form of folate needed to make DNA. If there is no B₁₂ available, this form of folate can become reduced (known as the methyl-folate trap) and DNA cannot be produced (13).

G. lamblia mostly colonizes in duodenum and jejunum, The intrinsic factor carries the B₁₂ to last section of the small intestine, the ileum, and the folate must be digested by pancreatic juice in the duodenum, therefore folic acid deficiency may cause malabsorption, because the folate digestion and *Giardia* colonization are in same place but vitamin B₁₂ deficiency is not absolutely related to *Giardia* colonization and more related to consume of this vitamin by *Giardia*.

The results of this experiment and previous (13, 20, 23) studies showed that vitamin B12 and folic acid not only reduced the pathogenesis of *G. lamblia* but also could replace the lost vitamins because of giardiasis. Then getting the vitamin B12 and folic acid is suggested as a complementary treatment for infected individuals in giardiasis.

Immunofluorescence studies showed that cytoskeletons that were composed of actin and myosin II rapidly reorganized in vegetative dictyostelium cells upon chemotactic stimulation by folic acid (24). Other investigators have shown that manipulation of temperature, divalent cation concentration, or actin-myosin function affects the binding of *Giardia* trophozoites to glass or plastic surfaces (25-26). Actin and myosin are effective agents in muscle structure of ventral disk of *Giardia* (25-26). In another study, results were similar and indicated a role for the actin-myosin system in attachment, with little evidence

of a role for the microtubule system. Both actin and microtubules have been found in the periphery of the ventral disk (27). Lab tests that record vitamin B₁₂ levels in sera and stool fat may also be recommended for diagnosis of *G. lamblia* (6). It was concluded that cobalamin and folic acid might reduce pathogenesis induced by *G. lamblia* and could be suggested as a complementary treatment for infected individuals.

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