THE HEMATOLOGICAL STATUS, PLASMA VITAMIN B_{12} AND FOLIC ACID LEVELS, AND INTESTINAL PATHOLOGY IN RATS INFECTED WITH *GIARDIA LAMBLIA*

Cheeraratana Cheeramakara[1], Apichart Nontprasert[2], Chutatip Siripanth[3], Wanyarat Tanomsak[1], Udomporn Chularerk[4], Pramualmal Sucharit[3] and Suvit Areekul[1]

[1]Department of Tropical Radioisotopes, Faculty of Tropical Medicine; [2]Department of Clinical Tropical Meidicine, Faculty of Tropical Medicine; [3]Department of Protozoology, Faculty of Tropical Medicine, Mahidol University; [4]Department of Parasitology, Faculty of Public Health, Mahidol University, Bangkok, Thailand

Abstract. The purpose of our study was to investigate the hematological status, vitamin B_{12} and folic acid absorption and intestinal pathology after *Giardia lamblia* infection in a rat model. Adult Wistar rats were assigned randomly to receive human giardia cysts orally in the amount of 5×10^5 or 1.0×10^6 cysts, or none in the controls. The results showed that all the rats injected with giardia cysts became infected. The cyst output in the infected rats varied considerably. In rats infected with 5.0×10^5 giardia cysts, the incubation period until cyst output was 10 days compared with 4 days in rats infected with the higher amount of 1.0×10^6 giardia cysts. The highest peaks for cysts output in these 2 groups were on days 4-33, which decreased gradually to days 40-58. The hematocrit and hemoglobin levels in the infected rats were statistically significantly lower than in the controls on days 16, 22, 33, and 37 post-infection ($p < 0.05$). A reverse relationship between giardia cyst output and hemoglobin concentration was found in the infected rats ($p = 0.05$). There were no significant differences in plasma vitamin B_{12} and folic acid levels between the infected rats and the control rats. No pathological changes were found in the small intestine of infected rats. These findings suggest that giardiasis did not affect the absorption of plasma vitamin B_{12} and folic acid but caused anemia in a rat model.

INTRODUCTION

Giardia infection is a common cause of food and water-borne diarrhea in non-sanitary communities. It can cause massive outbreaks due to the contamination of water supplies in many developing countries. Infections are common in children, particularly in child-care centers, backpackers, travellers, and homosexuals. Each year, acute diarrhea is in the top-ten diseases that cause the highest morbidity rate in Thailand (Division of Epidemiology, 1995-1999). This report did not specify the causative agents of such acute diarrhea. Reports of specific giardia infection levels in Thailand are not well documented. The clinical manifestations of giardia infection vary considerably, from asymptomatic to acute infection. Clinical signs related to enteric protozoan diseases commonly involve malabsorption, diarrhea, weight loss or growth retardation and anorexia (Hjelt *et al*, 1993). Chronic infection with *G. lamblia* is common and can probably develop into acute infection (Chester *et al*, 1985). Giardiasis may present with severe damage of the small intestine with subsequent effects on nutrient absorption (Oberhuber *et al*, 1997). Malabsorption of disaccharides is a main complication of giardiasis (Vega-Franco *et al*, 1987; Gendrel *et al*, 1992). However, many patients infected with *G. intestinalis* do not present with symptoms or damage to the small intestine (Farthing, 1996).

The objective of the study was to investigate the effects of giardia infection on hematocrit and hemoglobin concentrations, vitamin B_{12} and folic acid levels and intestinal pathological abnormalities in a rat model.

Correspondence: Cheeraratana Cheeramakara, Department of Tropical Radioisotopes, Faculty of Tropical Medicine, Mahidol University,420/6 Rajvithi Road, Bangkok 10400, Thailand.
Tel: 66 (0) 2643-5585; Fax: 66 (0) 2643-5585
E-mail: tmccr@mahidol.ac.th

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighing 230 - 250 g from the National Laboratory Animal Center, Mahidol University, Thailand were used in this study. All the rats were kept for 2 weeks before starting the experiment. They were housed 1 rat per cage with access to food and water ad libitum.

Twenty-five rats were assigned randomly to receive giardia cysts orally, either in the amount of 5.0×10^5 cysts (n = 5) or 1.0×10^6 cysts (n = 20). The cysts were injected directly to pharynx of the rats using a gastric gavage no.16. Another group received water without giardia cysts as a control (n = 20).

Measurement of giardia cyst output

Rat feces were collected and giardia cysts were counted daily. The sawdust was changed after feces collection. Giardia cyst output was measured using the Stoll dilution technique. Cyst output was presented as an amount of cysts per grams wet weight of feces.

Collection of blood samples

The rats were anesthesized by inhalation of ether before drawing blood. The blood was sampled at time intervals before and after giardia injection. A heparinized capillary tube was gently inserted into the retro-orbital plexus of the rat eye. When the wall of the plexus was punctured, blood flowed out into the capillary tube to the EDTA-coated tube. The whole blood was then centrifuged at 2,500 rpm for 10 minutes. The plasma was kept at -20ºC until used.

Determination of hematocrit (hct) and hemoglobin (hgb) levels

The blood in the capillary tube was spun down at 10,000 rpm for 5 minutes for hematocrit measurement. The unclotted blood in the EDTA-coated tube was mixed thoroughly for the measurement of the hemoglobin concentration. Hb concentration was determined using the cyanomethemoglobin method.

Determination of plasma vitamin B_{12} and folic acid levels

Plasma vitamin B_{12} levels were measured using a modified techniques of radioisotopes dilution and coated charcoal (Lau *et al*, 1965; Grossowiczc, 1975). Plasma folate levels were measured using a microbiological growth assay of the *Lactobacillus casei* (Waters and Mollin, 1961).

Pathological examination of the small intestine

The rats were anesthesized with 4% sodium chlorohydrate solution. They were then infected intracardially with 0.9% sodium chloride in the left ventricle, followed by a fixative formalin solution [containing 37% formalin, 100 ml; distilled water, 900 ml; monobasic potassium phosphate (KH_2PO_4), 4.0 g; dibasic potassium phosphate (K_2HPO_4), 6.5 g] and a sodium chloride solution under physiological pressure (approximately 120 mm of mercury or 5 ml/minute). The three parts of the small intestine: duodenum, jejunum and ileum, were removed and put in a fixative solution for one week before tissue embedding in paraffin. The tissues were sectioned at 4 μm thickness with a microtome (Spencer). The tissue sections were stained with hematoxylin and eosin and mounted with permount (Fisher Scientific) and a cover slip. Microscopic examination was performed for pathological damage to the intestine. The results were confirmed by a veterinary pathologist.

Statistical analysis

Parametric comparision of means of variables was performed using the paired and unpaired *t*-test. Correlation between variables was determined by Spearman correlation coefficients. P-values < 0.05 were considered as statistically significant.

RESULTS

Infection rate and cyst excretion pattern in the experimental rats

All the rats were free from other intestinal parasitic infections before starting the experiment. The giardia cysts were prepared from the stool of patients with giardiasis who were admitted to the Bangkok Hospital for Tropical Diseases, Faculty of Tropical Medicine. After the injection of the human giardia cysts orally, all the rats became infected; the cyst output varried (Fig 1). The percentages of infectivity in those injected with 5.0×10^5 giardia cysts and 1.0×10^6 giardia cysts were followed-up over time, as shown in Table 1. Most

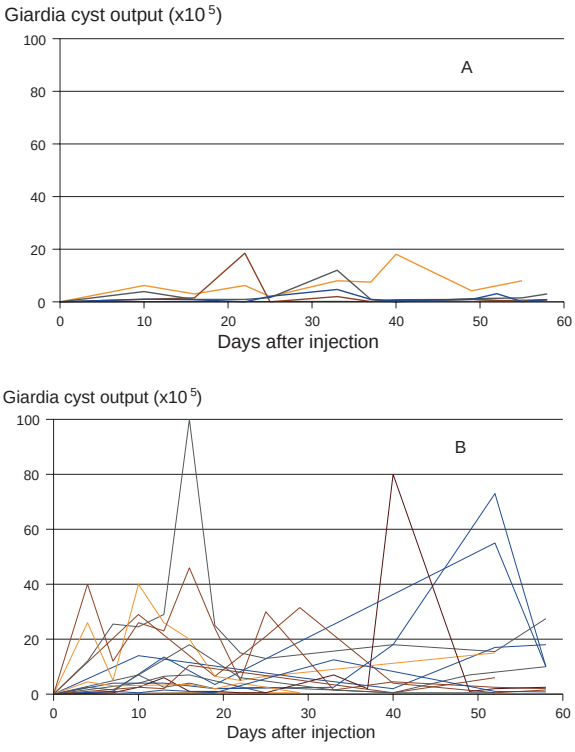

Fig 1–Cyst excretion pattern in the 5 rats exposed to 5.0 x 10^5 *Giardia lamblia* cysts (A) and 19 rats exposed to 1.0 x 10^6 giardia cysts (B) . The giardia cyst output is presented as the number of giardia cysts per gram of feces.

of the rats infected with 5.0 x 0^5 giardia cysts had cyst output on day 10 post-exposure (60 %) The latest day of no cyst output was on day 40 post-exposure (1/4). Some of these infected rats had no cyst output on days 22, 25, and 37 and post-exposure. The rats infected with 1.0 x 10^6 giardia cysts had earlier cyst output, day 4 post-exposure (32%). Only one of 20 rats had no cyst output until day 58 post-exposure. In other rats, no cyst output was observed on days 22, 25, 37, and 55 post-exposure. The peaks for cyst excretion in the 5.0 x 10^5 cysts-exposed group were on days 10, 22, 33, and 40 post-exposure compared to days 4, 10, 16, 25, and 40 post-exposure in 1.0 x 10^6 cysts-exposed group. Then, cyst excretion gradually declined. However, both of these groups still excreted cysts in feces until the end of the experiment (up to day 58 post-exposure).

Table 1
Infection rates in 5 rats exposed to 5.0 x 10^5 *Giardia lambia* cysts and 20 rats exposed to 1.0 x 10^6 *G. lambia* cysts.

Days after injection	Infection rates (%)	
	5.0 x 10^5 giardia cyst group	1.0 x 10^6 giardia cysts group
0	0 (0/5)	0 (0/19)
4	0 (0/5)	32 (6/19)
7	0 (0/5)	53 (10/19)
10	60 (3/5)	89 (17/19)
13	80 (4/5)	95 (18/19)
16	80 (4/5)	95 (18/19)
19	80 (4/5)	95 (18/19)
22	80 (4/5)	95 (18/19)
25	80 (4/5)	95 (18/19)
29	80 (4/5)	95 (18/19)
33	80 (4/5)	95 (18/19)
37	100 (5/5)	95 (18/19)
40	100 (5/5)	95 (18/19)
49	100 (5/5)	95 (18/19)
52	100 (5/5)	95 (18/19)
55	100 (5/5)	100 (19/19)
58	100 (5/5)	100 (19/19)

( ) is the ratio of infected rats to total rats.

Hematocrit and hemoglobin levels in rats

In the control group, the mean hct levels were lower than the initial baseline mean on days 7,10, and 13; the mean hgb levels on days 7, 10, 13, 19, 29, and 40 were lower than the initial baseline. The hct and hgb levels in the infected rats were significantly lower than the initial baseline from days 7 to 58 post-exposure. When comparing the infected rats with the control rats, the hct levels in the infected rats were significantly lower than the control rats on days 16, 22, 33, and 37 post-exposure ($p<0.025$-0.05), while the hgb concentrations in the infected rats were significantly lower than the control rats on days 13, 16, 22 and 37 post-exposure ($p<0.01$-0.05) (Fig 2).

Plasma vitamin B_{12} levels in rats

The plasma vitamin B_{12} levels in the infected rats (Fig 3) were not significantly different from the controls throughout the experiment ($p = 0.25$). After infection, the vitamin B_{12} levels in the in-

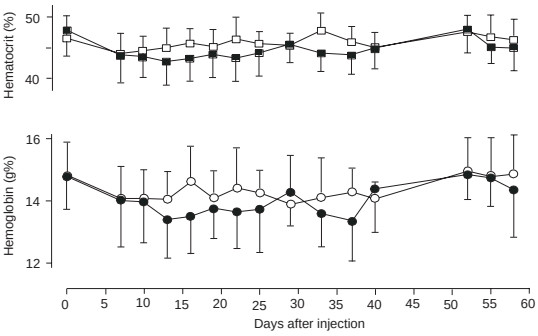

Fig 2–Comparison of hematocrit and hemoglobin levels between rats exposed to 1.0 x 10^6 *Giardia lamblia* cysts (dark symbols) and control rats (empty symbols). Vertical bars represent the mean ± one standard deviation.

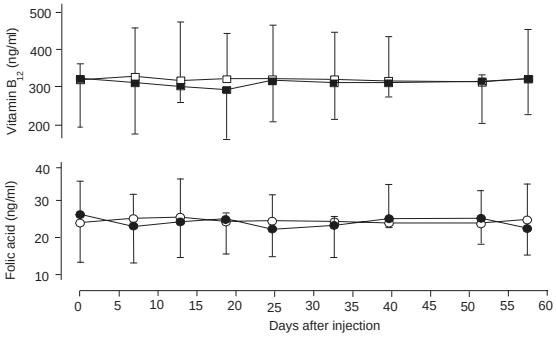

Fig 3–Comparison of plasma vitamin B_{12} and folic acid levels between rats exposed to 1.0 x 10^6 *Giardia lamblia* cysts (dark symbols) and control rats (empty symbols). Vertical bars represent the mean ± one standard deviation.

fected rats were lower than the baseline. In comparing the overall plasma vitamin B_{12} levels between 105 control samples and the 104 infected samples, the mean value for the vitamin B_{12} level in the infected rats (308 ± 123 pg/ml) was not significantly lower than the control rats (318 ± 123 pg/ml) ($p>0.05$).

Plasma folic acid levels in rats

The plasma folic acid levels in the infected rats (Fig 3) were not significantly different from the control rats ($p>0.05$). There was no significant difference between the overall plasma folic acid level from the 93 samples in the control group (23.71 ± 8.29 ng/ml) and the 97 samples in the infected group (23.36 ± 8.49 ng/ml) ($p>0.05$).

DISCUSSION

Giardiasis causes diverse clinical symptoms, from asymptomatic to acute and chronic diarrhea with or without malabsorption, in infected patients. The mechanisms by which giardia produces diarrhea and malabsorption and other symptoms are poorly understood. We have conducted this study to examine the hematological status, vitamin absorption, and intestinal pathology due to giardia infection in a rat model. In general, after injection of infective giardia cysts, cysts developed into trophozoites and inhabited the upper small intestine of the hosts. Giardia trophozoites may prevent nutrient absorption in the intestine or may cause intestinal lesions and subsequent nutritional malabsorption.

In this study, all the rats were successfully infected with human *G. lamblia* cysts. The cyst excretion pattern fluctuated and cyst output persistant until the end of the experiment. These findings are similar to those previously described (Roberts-Thompson *et al*, 1976; Belosevic and Faubert, 1983; Gillon and Ferguson, 1984). Craft (1982) found that the infection rate varied after the injection of 50 giardia cysts into rats. The incubation period continued until day 4 post-exposure. The infection peaked between days 7 and 20, however, spontaneous resolution of the infection occurred in 96% of the rats between days 28 and 42 post-exposure. Sehgal *et al* (1976) reported that only 5 out of 31 adult rats (16%) could be infected with 3,800-6,400 human giardia cysts during 10 days of observation. It may be that the cyst excretion in some rats was longer than 10 days. Robert-Thomson *et al* (1976) showed that mice challenged with 1,000 giardia cysts had a maximum cyst excretion rate after 1 to 2 weeks, then this progressively declined. This infection resolved spontaneously by 6 to 8 weeks. This may be explained by immunity to the infection. Simon (1922) was unable to infect 6 rats with 7.6 x 10^7 human giardia cysts suspended in milk, which the animals had fed on for 24 hours. The authors concluded that the giardia cysts were destroyed by the gastric juice of the animals.

The decrease in hct and hgb levels reveal that infection with giardiasis produces anemia in infected rats, which is in accordance with previous reports (Naik *et al*,1982; de Vizia *et al*, 1985). Naik *et al* (1982) reported a decrease in hgb concentration in 44 patients with giardiasis (13.4 g/dl) when compared to the 22 controls (14.3 g/dl). The most common presenting clinical manifestrations of giardia infection were diarrhea, anorexia, cramps, abdominal pain, and weight loss in 13 out of 17 patients (Hartong *et al*, 1979) and in all 5 patients with giardiasis (Ament and Rubin, 1972). This may suggest that giardiasis causes decreased food intake in these patients and subsequently produces anemia. De Vizia *et al* (1985) found that in 10 patients with giardiasis, 3 patients had a low body weight, 4 children had anemia with a hemoglobin concentration of 11.5 g/dl (two had iron deficiency at 3 months follow-up). After giardial treatment, 9 out of 10 patients had a prompt conversion to normal iron absorption. The authors concluded that iron deficiency was mainly related to the impaired absorption of iron in giardia infection.

No signifcant changes of vitamin B_{12} and folic acid levels were observed in rats infected with *Giardia lamblia*. Naik *et al* (1982) showed that patients suffering from symptomatic giardiasis did not have significant changes in serum vitamin B_{12} and folic acid levels; 213.51 pg/ml of vitamin B_{12} in 39 patients,161.00 pg/ml of vitamin B_{12} in 15 controls; and 11.02 ng/ml of folic acid in 43 patients, 9.44 ng/ml of folic acid in 15 controls. Subnormal serum vitamin B_{12} and folic acid levels were seen in 5 and 3 patients out of 19 patients, respectively. The urinary excretion of ^{57}Co-vitamin B_{12} in 19 patients was not significantly different from 13 normal subjects. There was no correlation between serum vitamin B_{12} and the intestinal absorption of vitamin B_{12} in both the groups: 13 controls and 19 patients. Determination of serum vitamin B_{12} and folic acid levels 4 weeks after the eradication of giardia infection showed no significant differences. Thus, *G. lamblia* could not be considered as a responsible agent for intestinal malabsorption of vitamin B_{12} and folic acid (Naik *et al*, 1982).

Some patients with giardiasis have developed malabsorption of vitamin B_{12} but recovered after drug treatment (Cowen and Campbell, 1973; Hartong *et al*, 1979; Egger *et al*, 1990). Twenty-nine out of 40 symptomatic patients (72%) in one report (Wright *et al*, 1977) with giardiasis developed histological abnormalities in the small intestine and vitamin B_{12} malabsorption. Improvement in absorption and jejunal morphology was observed after giardial treatment. In 6 patients with *G. lamblia* infection, one patient with general hypoproteinemia and hypogammaglobulinemia had folic acid deficiency and another one of 6 patients had a sprue-like syndrome with IgA deficiency and vitamin B_{12} malabsorption (Hoskins *et al*, 1967). This malabsorption of folic acid and vitamin B_{12} was in accordance with the results of a study involving 8 patients with hypogammaglobulinemia and gastrointestinal symptoms caused by *G. lamblia* infection (Ament and Rubin, 1972). Improvement of folic acid and vitamin B_{12} absorption appeared after giardia eradication.

Cowen and Campbell (1973) reported on radioactive vitamin B_{12} absorption in 3 patients with giardiasis before therapy (in plasma, 0.22%; in urine with intrinsic factor, 3.00%) and after therapy (in plasma, 0.64%; in urine with intrinsic factor, 11.3%) comparing to normal range (in plasma, 0.67-2.19 %; in urine with intrinsic factor >8%). They interpreted the results as malabsorption of vitamin B_{12}, which improved after elimination of the giardia infection. This was explained by the competition between the parasites and hosts for vitamin B_{12}. Hoskins *et al* (1967) reported that a lower level of serum folic acid was present in one patient (3.2 ng/ml; normal range, >7ng/ml) and serum vitamin B_{12} in another patient (<200 pg/ml; normal range, 200 to 900 pg/ml) from a total of 6 patients with giardiasis. The authors suggested this was due to either competition for dietary sources of these vitamins or malabsorption due to giardia infection. Hartong *et al* (1979) found low serum folic acid levels in 4 (4.45 ng/ml) out of 11 patients (7.42 ng/ml) and low vitamin B_{12} levels in 6 (6.85%) out of 10 patients (16.5%) with giardiasis. Vitamin B_{12} absorption was found to be 8.6% in patients with giardiasis and 16.6% after treatment for giardiasis. The author suggested that morphological abnormalities of the small intestinal mucosa may account for changes in the absorptive function, and other factors, such as bacterial overgrowth and exocrine

pancreatic insufficiency may also contribute to malabsorption in some patients with giardiasis.

Malnutritional due to poor absorption with giardia infection is still controversial. For histopathological examination of intestine found that *G. muris* caused small intestinal injury and interfered with nutrient absorption in infected mice (Roberts-Thompson *et al*, 1976; Gillon and Ferguson,1984; Buret *et al*, 1990). In the duodenum and jejunum, where trophozoites were most commonly found; villus atrophy and brush border enzyme deficiency was noted. In our study, we could not find any malabsorption of vitamin B_{12} or folic acid or intestinal damage, in spite of the large number of giardia cysts. Malabsorption of nutrients may occur when animals have intestinal infestation for a long time and having intestinal damage. In summary, giardia infectin in a rat model did not cause vitamin malabsorption or intestine damage, but did cause anemia.

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