Ethanol Effects on Intestinal Functions in *Giardia lamblia*-Infected Rats

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Summary The effect of *Giardia lamblia* infection on D-glucose uptake and brush-border enzymes was studied in chronically ethanol-fed rats. *Giardia lamblia* trophozoites counts were significantly lower in the intestine of ethanol-fed rats than in the controls. Also, the Na⁺-dependent uptake of D-glucose and brush-border enzymes was significantly reduced ($p < 0.0001$, $p < 0.01$, respectively) in the *G. lamblia*-infected rat intestine. There was no change in Na⁺-independent glucose uptake under these conditions. Levels of mRNA encoding Na⁺-dependent D-glucose transporter (SGLT1) and brush-border sucrase and lactase were reduced in *G. lamblia*-infected animals compared with those of the controls. However, the mRNA levels encoding these proteins in ethanol-fed and control animals were similar in rat intestine. These findings suggest that down-regulation of SGLT1 and disaccharidases could be responsible for the observed D-glucose malabsorption and decrease in the activity of disaccharidases in *G. lamblia* infection.

Key Words: *Giardia lamblia*, ethanol feeding, malabsorption, sugar transport (D-glucose), disaccharidases

Giardia lamblia infection is a known cause of acute and chronic intestinal dysfunctions [1–3]. However, the factors augmenting this parasitic infection are not known. The prerequisite for colonization of the parasite in the intestine is the adhesion of trophozoites to the epithelial cell surface [4]. It is amply recognized that surface oligosaccharides are implicated in the adhesion of microflora to the

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cell surface [5]. The surface of *Giardia* trophozoites contains a carbohydrate binding protein that recognizes *N*-acetylglucosamine/sialic acid; thus, lectin-mediated receptor binding has been proposed as the mechanism of attachment of this protozoa to the intestinal epithelium [6, 7]. We previously reported that chronic ethanol feeding markedly altered the glycosylation pattern in the rat intestine [8]. The sialic acid content of brush borders was markedly enhanced with a concomitant decrease in fucose levels in response to ethanol administration [8]. The observed glycosylation pattern in ethanol-fed animals is similar to that observed in suckling rats [9], which are more prone to *Giardia* infection [10]. Thus to examine the role of ethanol ingestion in the pathogenesis of giardiasis, the present studies were undertaken to investigate the effect of *G. lamblia* infection on intestinal functions in chronically ethanol-fed rats. In addition, the mRNA levels encoding the Na⁺-dependent D-glucose transporter (SGLT1) and brush-border sucrase and lactase enzymes were also analyzed under these conditions.

MATERIALS AND METHODS

Animals and treatments. Male Wistar rats (30–40 g body wt) were used. Animals were divided into two groups. The animals in group I were administered orally 1 ml of 30% ethanol daily via a Ryle's tube for 4 weeks. Rats in group II received an isocaloric solution of glucose.

G. lamblia cysts obtained from human stool were purified on a sucrose gradient [11]. Inoculum dose of 10,000 cysts/animal in 0.2 ml of normal saline was given orally via a Ryle's tube to half of the unanesthetized animals in both group I (ethanol fed) and group II (isocaloric glucose fed).

Preparation of brush-border membranes. Overnight fasted rats were sacrificed under ether anesthesia. Starting from the ligament of Treitz, 20–25 cm of intestine was removed and thoroughly washed with ice-cold saline. Brush-border membrane (BBM) was isolated and purified following the method of Schmitz *et al.* [12].

Enzyme assays. Brush-border sucrase or lactase activities were assayed following the method of Dahlquist [13]. Alkaline phosphatase and leucine aminopeptidase activities were determined according to the method of Bergmeyer [14] and Naftalin *et al.* [15], respectively. Protein was estimated by the method of Lowry *et al.* [16], with bovine serum albumin used as the standard.

Quantification of trophozoites. The small intestine of the infected rats was removed on day 10 postinfection and flushed with a fixed amount of normal saline. The trophozoites were counted in a hemocytometer, and the results were expressed as total number of trophozoites in the drained fluid.

D-Glucose uptake measurement. The uptake of D-glucose was studied by use of radiolabeled [U-¹⁴C]D-glucose, as described previously [17]. Everted intestinal segments (0.3–0.5 cm) were incubated for 5 min at 37°C in 5 ml of oxygenated (95% O₂-5% CO₂) Tris-maleate buffer (120 mM NaCl or choline chloride, 1.21 mM

MgSO₄, 0.63 mM CaCl₂ and Tris-maleate solution of pH 5.5 and 7.2 to bring the final osmolarity to 300 mOsm). The buffer contained 5 mM D-glucose with a trace amount of [U-¹⁴C]glucose (sp. activity: 160 mCi/mmol). After incubation, tissues were gently blotted on filter paper, weighed, and digested in 10% KOH. The radioactivity was determined in an LKB Reck Beta Scintillation counter. The uptake rate was corrected for the extracellular space measured with [³H]inulin and expressed as $\mu\text{mol glucose}/5 \text{ min/g tissue}$.

RNA preparation and Northern blot analysis. Total RNA from the intestine frozen in liquid N₂ was isolated by the guanidinium thiocyanate method [18] and resolved on formaldehyde-agarose gels [19]. The denatured RNA was transferred onto nylon membranes (zeta probe, Bio-Rad, Hercules, CA) by a capillary transfer method using 50 mM NaOH [20]. The transferred RNA was fixed on membranes by exposing the membrane carrying RNA to a source of low doses of ultraviolet irradiation (strata-UV crosslinker). Prehybridization of the membrane containing immobilized RNA was carried in SDS formamide buffer at 42°C for 4 h [20]. Simultaneously the oligonucleotide probes were added to the pre-hybridization sealed bags and hybridized for 20–24 h at 42°C. After hybridization, the membranes were washed with 2 × SSC, 0.1% SDS (20 min, each three times) at 42°C and autoradiographed by exposing the filter to X-ray film (Kodak XAR-2) for 3–5 days at –70°C with an intensifying screen. The antisense oligonucleotide probes complementary to mRNA were used. The oligonucleotide probes used were 5' 249–273 for Na⁺-glucose transporter [21], 5' 13–33 for lactase [22], and 5' 92–119 for sucrase [23]. β -Actin probe was used as a marker of a housekeeping gene.

Statistical analysis of data was done by Student's *t*-test and analysis of variance (ANOVA).

RESULTS

G. lamblia-infected animals manifested watery diarrhea, which was maximal on day 10 postinfection. The trophozoites counted in the intestinal flush out of these animals revealed a high degree of infection in both glucose-fed and ethanol-fed rats exposed to *G. lamblia* (Table 1). Interestingly, the number of *G. lamblia*

Table 1. Intestinal uptake of D-glucose in control and ethanol-fed rats infected with *G. lamblia*.

Parameter	Control		Ethanol fed	
	(–) <i>G. lamblia</i>	(+) <i>G. lamblia</i>	(–) <i>G. lamblia</i>	(+) <i>G. lamblia</i>
Trophozoite counts (log ₁₀)	Nil	8.39 ± 3.74	Nil	7.26 ± 2.58***
D-Glucose uptake [#] ($\mu\text{mol glucose}/5 \text{ min/g tissue wt}$)				
(+) Na ⁺	5.74 ± 0.98	3.65 ± 0.35*** ^a	6.37 ± 0.76	4.15 ± 1.47*** ^b
(–) Na ⁺	2.37 ± 0.20	2.02 ± 0.61	2.55 ± 0.23	3.31 ± 0.81

Values are mean ± SD of 4–6 observations in each group. Significant differences*** $p < 0.001$ compared with controls by Student's *t*-test. ^a Compared to control (–) *G. lamblia*. ^b Compared to ethanol (–) *G. lamblia*. [#] Significant according to ANOVA.

Table 2. Effect of ethanol feeding and *G. lamblia* infection on brush-border enzymes in rat intestine.

Enzyme	Control		Ethanol fed	
	(-) <i>G. lamblia</i>	(+) <i>G. lamblia</i>	(-) <i>G. lamblia</i>	(+) <i>G. lamblia</i>
Sucrase [#]	1.13±0.19	0.26±0.09*** ^a	0.67±0.06	0.28±0.07*** ^{a,b}
Lactase [#] (units/g protein)	77.4±10.1	61.7±11.0* ^a	81.9±11.1	65.3±7.08* ^{a,b}
Alkaline phosphatase [#]	1.10±0.27	0.38±0.01*** ^a	0.58±0.07*** ^a	0.50±0.14*** ^a
Leucine aminopeptidase [#]	0.45±0.10	0.23±0.02*** ^a	0.34±0.04	0.24±0.11* ^a

Values are mean±SD of 4-6 observations as units/mg protein. 1 unit is equal to 1 μ mol of substrate hydrolyzed per minute under standard assay conditions. Significant differences * p <0.05; ** p <0.01; *** p <0.001 compared with controls by Student's *t* test. ^aCompared to control (-) *G. lamblia*. ^bCompared to ethanol (-) *G. lamblia*. [#]Significant according to ANOVA.

Fig. 1. Northern blot analysis of mRNA by agarose gel electrophoresis (a). Total RNA from small intestine of control and ethanol-fed rats infected with *G. lamblia* was obtained by guanidinium thiocyanate and phenol-chloroform extraction and analyzed on 1% agarose gels stained with ethidium bromide. Northern blot analysis of mRNA encoding β -actin (b). Each lane was loaded with 10 μ g of total RNA. Lane 1, Control (-) *G. lamblia*; lane 2, control (+) *G. lamblia*; lane 3, ethanol-fed (-) *G. lamblia* and lane 4, ethanol-fed (+) *G. lamblia*.

trophozoites was significantly lower ($p < 0.001$) in the ethanol-fed rats than in the glucose-fed controls (group I). There was no change in Na^+ -independent D-glucose uptake ($2.02\text{--}3.31 \mu\text{mol glucose}/5 \text{ min/g tissue}$ in ethanol-or fed *G. lamblia*-infected animals compared with that in the uninfected controls. Feeding of ethanol to rats did not affect Na^+ -dependent D-glucose uptake ($5.74\text{--}6.37 \mu\text{mol glucose}/5 \text{ min/g tissue}$ from the intestine. However, Na^+ -dependent sugar uptake was significantly ($p < 0.001$) reduced in *Giardia*-infected rats compared with that in uninfected controls (Table 1).

Ethanol feeding for 4 weeks to rats significantly reduced the activities of sucrase, alkaline phosphatase (AP), and leucine amino peptidase (LAP) compared with the control levels (group I). However, there was no change in lactase activity under these conditions. The activities of brush-border sucrase, lactase, AP, and LAP were markedly reduced in *G. lamblia*-infected animals fed isocaloric glucose or ethanol compared with those of non-infected animals (Table 2).

As shown in Fig. 1, there was no change in mRNA levels encoding β -actin used as a housekeeping gene, in the control, ethanol-treated or *Giardia*-infected animals. Northern blot analysis, however, revealed a marked decrease in mRNA levels encoding sucrase, lactase, and Na^+ -dependent D-glucose transporter (SGLT1) in *G. lamblia*-infected rat intestine compared with the levels for the

Fig. 2. Northern blot analysis of mRNA encoding brush-border sucrase (a) and lactase (b) in control and ethanol-fed rats infected with *G. lamblia*. Each lane contained $10 \mu\text{g}$ of intestinal RNA. Blots were hybridized with radiolabeled oligonucleotide probes. Autoradiography was carried using X-ray film (Kodak XAR-2) and intensifying screens at -70°C for 3–5 days. Lane 1, control (–) *G. lamblia*; lane 2, control (+) *G. lamblia*; lane 3, ethanol-fed (–) *G. lamblia*, and lane 4, ethanol-fed (+) *G. lamblia*.

**GLUCOSE TRANSPORTER
(SGLT1)**

Fig. 3. Northern blot analysis of mRNA encoding Na^+ -dependent D-glucose transporter in control and ethanol-fed rats infected with *G. lamblia*. Each lane contained $10\text{ }\mu\text{g}$ of intestinal RNA from small intestine. Blots were hybridized with radiolabeled oligonucleotide probe. Autoradiography was carried out using X-ray film (Kodak XAR-2) and intensifying screens at -70°C for 3–5 days. Lane 1, control (–) *G. lamblia*; lane 2, control (+) *G. lamblia*; lane 3, ethanol-fed (–) *G. lamblia* and lane 4, ethanol-fed (+) *G. lamblia*.

control group. However, the mRNA levels were essentially similar in ethanol-fed and control (group I) rats. Levels of mRNA encoding disaccharidases or SGLT1 cotransporter in *Giardia*-infected animals were essentially similar to those in ethanol-fed, *Giardia*-exposed animals. Thus ethanol administration *per se* did not affect the mRNA levels of the enzyme and the glucose- Na^+ co-transporter in the rat intestine. But *G. lamblia* infection induced a significant decrease in the amounts of the mRNA transcripts. Radiolabeled probes of sucrase and lactase hybridized with 6.5-kb (Fig. 2a) and 6.8-kb (Fig. 2b) transcripts, respectively. The SGLT1 probe hybridized to two transcripts of 4.5-kb and 2.8-kb fragments (Fig. 3). The 2.8-kb transcript was only of faint intensity. This is due to the fact that the two mRNA fragments contain the same coding region with different length of the 3' non-coding region [24]. Therefore, the observed decrease in brush-border sucrase and lactase activity as well as in D-glucose uptake in *G. lamblia*-infected rat intestine is a consequence of the down-regulation of the gene expression of these proteins.

DISCUSSION

The results presented here indicate lower levels of *G. lamblia* trophozoites in the intestine of ethanol-fed rats than in that of the control animals. Farthing *et al.* [7] suggested the presence of WGA-like lectin on the surface of *G. lamblia* cyst to be responsible for the adhesion of the parasite to the luminal surface of enterocytes.

Thus, the observed decrease in *G. lamblia* counts in ethanol-fed animals could be attributed to a modified glycosylation pattern of enterocytes under these

conditions [25]. The low levels of the parasite in the ethanol-fed intestine, however, can not be due to a direct effect of ethanol on the *Giardia* trophozoites, for during the period of infection, the animals were not exposed to ethanol. The indirect effects of the ethanol ingestion, as a consequence of changes in biliary and pancreatic secretions in the gut, however, can not be ruled out. But, nevertheless, the finding indicating lower counts of *Giardia* trophozoites in ethanol-fed rats than in those given isocaloric glucose is interesting and needs further investigation.

The present data also indicate that *G. lamblia* infection in both the control and chronically ethanol-fed animals induced a similar decrease in D-glucose uptake and brush-border enzymes, although there was a significant difference in the degree of parasitemia in the two groups. The observed decrease in sucrase, lactase, AP, and LAP activities in *G. lamblia*-infected rats was similar to that reported earlier [26]. A similar decrease in D-glucose uptake from the intestine in animals exposed to *G. lamblia* has also been reported [26]. Both giardiasis and ethanol feeding are known to produce morphological alterations in the intestine [27, 28]. The mechanism of intestinal malabsorption in giardiasis is unknown, although a number of hypotheses have been advanced to explain this phenomenon, such as (a) mechanical obstruction of the villus surface by abundance of the *G. lamblia* trophozoites [27]; (b) damage of the intestinal mucosa as a sequel to the parasitic infection; (c) presence of bacterial overgrowth or fungal growth; (d) invasion of the epithelial cells by *Giardia* infection; (e) changes in the turnover rate of epithelial cells; (f) enhanced secretion of mucus or mucoid substances in the presence of parasites, as has been observed in humans and in experimental models [29]. It is likely that a combination of these factors could be implicated in the pathogenesis of intestinal dysfunctions in giardiasis.

In the present studies, low levels of mRNA encoding for D-glucose transporter and brush-border sucrase and lactase activities were demonstrated in the *G. lamblia*-infected rat intestine. However, there was no change in mRNA levels of these proteins in chronically ethanol-fed rats. This apparently indicates that the underlying mechanisms of malabsorption in giardiasis and chronic alcoholism are distinct. Presumably, ethanol feeding affects the cell morphology leading to aberration of surface enterocytes. However, *G. lamblia* infection induces malabsorption by affecting the expression of brush-border disaccharidases and sugar transporter proteins. The SGLT1-specific oligonucleotide probe hybridized with 4.5-kb and 2.8-kb fragments of intestinal mRNA. Coady *et al.* [30] earlier reported homology between cDNA clones of Na⁺-glucose cotransporter in human and several animal species. The rabbit intestinal Na-glucose cotransporter cDNA used by them hybridized strongly with 4.5-kb and weakly with 2.8-kb mRNA transcripts. This is identical to the findings described herein. This could be an additional mechanism of intestinal malabsorption in this infection.

In conclusion, the present findings indicate that *G. lamblia* counts were decreased in intestine of ethanol-fed rats; which is presumably due to alteration in glycosylation pattern in intestinal epithelium in these animals [8]. *G. lamblia*

infection induced a marked decrease in D-glucose uptake and in the activities of various brush-border enzymes, which was as a consequence of down-regulation of the expression of mRNA encoding these proteins. Ethanol feeding, however, had no effect on the expression of these proteins in the rat intestine.

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