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Research Article

EFFECTS OF ETHANOL DURING GIARDIASIS IN SHEEP INTESTINE

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ABSTRACT
Infections with *Giardia lamblia* are one of the most common intestinal maladies in the world. These infections can lead to acute diarrhea, cramps, and nausea, although asymptomatic infections are the most common. Although most infections are controlled by an effective immune response, some individuals develop chronic disease. The effects of Giardia lamblia infection on D-glucose uptake and brush border enzymes was studied in ethanol fed sheep. Giardia lamblia trophozoite counts were significantly lower in the intestine of ethanol fed sheep than in the controls. Also sodium dependant uptake of D-glucose and brush border enzymes was significantly reduced in the Giardia lamblia infected sheep intestine. There was no change in sodium dependent D-glucose transporter (SGLT-1) and brush border lactase was reduced in Giardia lamblia infected sheep compared with those of controls. However, the mRNA levels encoding these proteins in ethanol fed animals and control animals were in the sheep intestine. The D-glucose malabsorption was observed and probably it causes a significant decrease in activity of disaccharidases in Giardia lamblia infection.

KEY WORDS: Giardia lamblia, Malabsorption, Sugar Transport and Brush border enzymes.

INTRODUCTION

Infections with *Giardia lamblia* are one of the most common intestinal maladies in the world[1]. These infections can lead to acute diarrhea, cramps, and nausea, although asymptomatic infections are the most common. Although most infections are controlled by an effective immune response, some individuals develop chronic disease. The requirement for colonization of the parasite in the intestine is the adhesion of trophozites to the epithelial to the epithelial cell surface[2]. Surface oligosaccharides are implicated in the adhesion of microflora to the cell surface[3]. The surface of Giardia lamblia trophozites 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[4] to the intestinal epithelium. Chronic ethanol feeding markedly altered the glycosylation pattern[5] in the rat intestine. The sialic acid content of the brush borders was markedly enhanced with a decrease in fucose in response to ethanol fed animals, which are prone to Giardia lamblia infection. The present studies were undertaken to investigate the effects of Giardia lamblia infection on intestinal functions in ethanol fed sheep. In addition, the mRNA levels encoding the sodium dependant D-glucose transporter (SGLT-1) and brush border lactase enzyme was analyzed under these conditions. With the advent of new biochemical, molecular, and genetic techniques, there has been considerable activity in characterizing *Giardia* speciation. Meloni[6] et al. described the isoenzyme electrophoresis analysis studies[7-8] and recombinant DNA probe characterizations[9-10], which have been conducted with *Giardia* to attempt to characterize the speciation. The Merifluor antibody staining was used to retrospectively evaluate human stool specimens[11]. A different antibody kit, *Giardia*-CEL (Cellabs P.L., Sydney, Australia), was utilized to compare phase contrast microscopy and the DFA assay for detecting *Giardia* cysts in cattle and wild rodent feces[12]. Of 40 cattle fecal specimens examined, 31(78%) were positive by DFA and only 17 (55%) were positive by phase contrast microscopy. All of the detected cysts VII-28 were identified as belonging to the *G. duodenalis* group[13]. *Giardia* was found in 103/216 (48%) fecal specimens collected from wild rodents. There is no published information on the occurrence of *Giardia* in soils and sediments, probably due to the difficulty in examining this type of sample for cysts. Methods are needed for detecting, identifying and enumerating cysts in soils and sediments. After suitable methods are developed and evaluated, they should be applied in laboratory and field studies to determine the persistence of *Giardia* in these media.

MATERIALS AND METHODS

Animals And Treatment: Male sheep of 1.5 years age, having average body weight, were used. Animals were divided into two groups. Group-I: Daily Oral administration of 30ml of 25% ethanol for one month. Group-II: Isocaloric solution of glucose was given orally.

Giardia lamblia cysts were obtained from human stool were purified on a sucrose gradient. Inoculum dose of 10,000 cysts/ animal in 20ml of normal saline was given orally half of the unanesthetized animals in both Gp.I (ethanol fed) and Gp.II (Isocaloric glucose fed).

Preparation Of Brush Border Membranes: Overnight fasted sheep were sacrificed under ether anesthesia. Starting from the ligament of Treitz, 20-35 cm of intestine was removed and thoroughly washed with ice cold saline. Brush border membrane (BBM) was isolated and purified[14].

Enzyme Assays: Brush border lactase was assayed following the method of Dahlquist[15]. Alkaline phosphatase and leucine amino peptidase activities were determined according to the method of Bergmeyer[16]. Protein was estimated[17] with bovine serum albumin used as the standard. The small intestine of the infected sheep was removed on day 7th post infection and flushed with a normal saline. The trophozites were counted in a hemocytometer and expressed as total number of trophozites in the drained fluid.The uptake of D-glucose was studied by use of radiolabelled [U-14C] D-glucose[18]. Everted intestine segments (1.5-2.5cm) were incubated for 3 min. at 37^{0}C in 5 ml of oxygenated (95% O_2, 5% CO_2) Tris maleate buffer. The buffer contained 5mM D-glucose with trace amount of U-14C glucose (specific Activity 160mCi/mmol)[19]. After incubation tissues were blotted on filter paper weighed and digested on 10% KOH. The radio

activity was determined in a β Scintillation counter[18]. The uptake rate was determined corrected for the extracellular space measured with [^{3}H] inulin and expressed as μmol glucose/min/gram tissue.

RNA Preparation And Northern Blot Analysis: Total RNA from the intestine frozen in liquid nitrogen was isolated by the Guanidinium thiocyanate method[20] and resolved on formaldehyde-agarose gels. The denatured RNA was transferred onto nylon membranes using 50mM NaOH. The transferred RNA was fixed on membranes by exposing the membrane carrying RNA to a source of low doses of UV irradiation. Prehybridization of the membrane containing immobilized RNA was carried in sodium dodecyl sulphate-formamide buffer at 40^{0}C for 3hours[21]The oligonucleotide probes were added to the pre-hybridization sealed bags and hybridized for 24h at 40^{0}C. Membranes were washed with 1%SDS and autoradiographed by exposing the filter to X-ray film (Konica) for 3 days at -50^{0}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[22], 5'13-33 for lactase[23]. β actin probe was used as marker of a house keeping gene.

Statistical analysis was done by student t-test and analysis of variance.

RESULTS AND DISCUSSIONS

Giardia lamblia infected animals manifested watery diarrhea which was maximal on day 7th post infection. The trophozites counted in the intestinal flush out of these animals revealed a high degree of infection in both glucose fed and ethanol fed sheep exposed to Giardia lamblia.(Table). The number of Giardia lamblia trophozites was significantly low ($p<0.001$) in the ethanol fed sheep than in the glucose fed controls (Gp I). There was no change in sodium independent D-glucose uptake (3.20-4.61μmol glucose/1min/gm tissue in ethanol or Giardia lamblia infected animals compared with that in the uninfected controls. Feeding of ethanol to sheep did not affect sodium dependent D-glucose uptake (7.74-9.14 μmol glucose/1min/gm tissue) from the intestine. However, sodium dependent sugar uptake was significantly ($p<0.001$) reduced in Giardia lamblia infected sheep compared with that in uninfected controls. Ethanol feeding for a month to sheep significantly reduced the activity of alkaline phosphatase (AP) and leucine amino peptidase (LAP) compared with the control (Gp.I). However, there was no change in sucrase activity under these conditions. The activities of brush border lactase, Alkaline Phosphatase and Leucine Amino Peptidase were distinctly reduced in Giardia lamblia–infected animals fed isocaloric glucose or ethanol compared with those of animals. As shown, there was no change in mRNA levels encoding β-actin used as a housekeeping gene, in control, ethanol-treated or Giardia lamblia-infected animals. Northern blot analysis, revealed a marked decrease in mRNA levels encoding lactase, and sodium dependent D-glucose transporter (SGLT1) in Giardia lamblia-infected sheep intestine compared with the levels for the control group. Levels of encoding disaccharidases or SGLT1 cotransporter in Giardia lamblia-infected animals were essentially similar to those in ethanol-fed Giardia lamblia exposed animals. Thus ethanol administration as such, did not affect the mRNA levels of the enzyme and the glucose-Sodium-co-transporter in the sheep intestine. But Giardia lamblia infection induced a significant decrease in the amount of mRNA transcripts. Radiolabelled probes of lactase hybridized with 6.5kb and 6.8kb transcripts, respectively. The SGLT1 probe hybridized to two transcripts of 4.5kb and 2.8kb fragments (Figure). The 2.8kb transcript was of faint intensity. The observed decrease in brush border lactase activity in D-glucose uptake in Giardia lamblia infected sheep intestine is a consequence of down regulation of gene expression of the proteins. The results presented here indicate low levels of Giardia lamblia trophozites in the intestine of ethanol fed sheep than in that of the control animals. The observed decrease in Giardia lamblia counts in the ethanol fed animals could be attributed to modified glycosylation pattern of enterocytes under these conditions. The findings indicating poorer counts of Giardia lamblia trophozite in ethanol fed sheep than in those given isocaloric glucose is remarkable and requires further studies. There occurs homology between cDNA clones of sodium glucose co-transporter in human and several animal species[24-26].

The present data also indicate that Giardia 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 decrease in the degree of parasitemia in the two groups. A decrease in lactase, Alkaline Phosphatase, and Leucine amino peptidase activities in Giardia lamblia infected sheep was observed. A similar decrease in D-glucose uptake from the intestine in rats exposed to Giaradia lamblia has also been reported[27]. Both giardiasis and ethanol feeding are known to produce morphological alterations in the rat intestine. It is likely that a various factors could be implicated in the pathogenesis of intestinal dysfunctions in giardiasis[28-30].In the present studies, short levels of mRNA encoding for D-glucose transporter and brush birder lactase activities were demonstrated in the Giardia lamblia infected sheep intestine. However, there was no change in mRNA levels of these proteins in chronically ethanol fed sheep. This apparently indicates that 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, Giardia lamblia infection induces malabsorption by affecting the expression of brush border disaccharidases and sugar transporter proteins. The SGLT1-specific oligonucleotide probe hybridized with 4.5kb and 2.8kb fragments of intestinal mRNA. The rabbit intestinal sodium glucose cotransporter cDNA used by them hybridized strongly with 4.5 kb and weakly with 2.8 kb mRNA transcripts. In conclusion, the present study indicates that Giardia lamblia counts were decreased in the intestine of ethanol fed animals, which is presumably due to alteration in glycosylation pattern in intestinal epithelium in these animals[5,29]. Giardia lamblia infection induced a marked decrease in D-glucose uptake and in the activity of various brush border enzymes, which was 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 sheep intestine.

RECOMMENDATIONS FOR ADDITIONAL RESEARCH: Additional information can assist in identifying and controlling risks of *Giardia* infection among children in day-care settings. With the increased globalization of our food supply, more surveillance of domestic and imported foods should be conducted in order to develop data for use in risk assessments and to ensure against outbreaks. The first successful Giardia vaccine, if one is developed, will probably be used in humans. Many questions related to the

host-parasite biology of *Giardia* remain. Further research is needed to help answer all of these questions.

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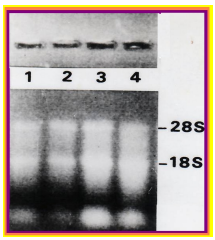
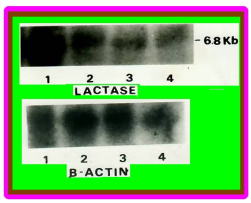
Figure 1 

Figure 2 & 3. 

Legends: Figure 1. Northern Blot Analysis of mRNA by agarose gel electrophoresis : Northern blot analysis of mRNA encoding β-actin. Lane 1, Control (-) G.lamblia; Lane 2, control (+) G. lamblia; Lane 3, Ethanol fed (-) G.lamblia and Lane 4, ethanol fed (+) G.lamblia.

Figure 2: Northern Blot Analysis of mRNA encoding brush border lactase in control and ethanol fed sheep infected with G.lamblia. Each lane contained 10µ of intestinal RNA.

Figure 3: Northern Blot Analysis of mRNA encoding sodium dependent D-glucose transporter in control and ethanol fed sheep infected with G.lamblia. Each lane contained 10µg of intestinal RNA from small intestine .

Table: Status of Intestine, D-glucose in ethanol fed & control sheep infected with G. lamblia

Parameter	Control		Ethanol Fed	
	(-) G.lamblia	(+) G.lamblia	(-) G.lamblia	(+) G.lamblia
Trophozite Counts	Nil	10.46 ±4.28	Nil	9.45± 2.13 ***a
(+) Na+	6.43±1.25	4.21±0.56***a	7.32±0.89	5.16±1.92***b
(-) Na+	2.94±0.68	3.03±0.72	3.62±0.57	4.24±1.2
LACTASE #(units /gm protein)	81.4±13.3	71.6 ±14.2*i	86.4± 14.1	69.5± 8.8 *i,ii
ALKALNE PHOSPHATASE #	1.43±0.38	0.56±0.07	0.76±0.05	0.65±0.19**i
LEUCINE AMINO PEPTIDASE#	0.64±0.15	0.29±0.03**i	0.42±0.08	0.26±0.92*i

Values are mean ± SD as units/mg protein.
Significant differences ***p<0.001; compared with controls by student's t-test; # Significant according to ANOVA.
a.- compared to control (-) Giardia lamblia; b. compared to control (+) Giardia lamblia.

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