Expression of sodium-glucose co-transporter and brush border disaccharidases in *Giardia lamblia* infected rat intestine

S Mahmood*, C P Sodhi and N K Ganguly
Department of Experimental Medicine & Biotechnology, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India

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The absorption of D-glucose and brush border membrane disaccharidases in the intestine of rat during infection by *Giardia lamblia* has been studied. The level of mRNA encoding Na⁺/glucose co-transporter (SGLT1) and brush border sacrase and lactase activities were also analyzed. At the peak of infection, i.e., day 7, 11 and 15 post-infection, there was a marked decrease in the signal of 4.5 kb and 2.8 kb mRNAs encoding SGLT1 compared to the controls. A similar decrease in sacrase and lactase mRNA’s (6.5 kb and 6.8 kb respectively) was also observed under these conditions. This corresponds to observed decrease in the rate of Na⁺-dependent D-glucose uptake and low activities of brush border sucrase and lactase under these conditions. There was no change in Na⁺-independent D-glucose uptake in giardia infected rat intestine. These findings suggest that the down regulation of the expression of SGLT1 and brush border sucrose and lactase activities may be responsible for the observed malabsorption in *G. lamblia* infection.

*Giardia lamblia* is a protozoal parasite which affects the structure and function of small intestine. Studies with animal models and in humans have shown that with *G. lamblia* infection causes a variety of biochemical, morphological and functional derangements in the gut resulting in diarrhoea and malabsorption. Anand et al. described a marked reduction in the levels of brush border enzymes in *Giardia* infected rats. Electron microscopic studies have shown structural damage to intestinal epithelial cell surface in giardiasis. It is amply recognized that the transport of D-glucose occurs by Na⁺-coupled co-transport system (SGLT1) in intestine. SGLT1 and sodium coupled glucose transport system 2 (SGLT2 found in kidney cortex) two closely related transport proteins have been cloned and their complete sequence of cDNA has been determined. A strong homology between cDNA clones of intestinal Na⁺-glucose co-transporters among various animal species has been demonstrated. Among many etiological factors responsible for diarrhoea and intestinal malabsorption are the enteropathogens, which affect intestinal function by causing changes in cell surface/or by modifying gene expression of brush border enzymes and transporter proteins located in the microvillus membrane. The present study was undertaken to evaluate the effect of *G. lamblia* infection on the expression of SGLT1 and brush border disaccharidases in rat intestine.

**Materials and Methods**

**Animals and mode of infection**

Albino rats (Wistar strain) three weeks old and free of intestinal parasites were used. A group of 20 rats was infected with *G. lamblia* cysts obtained from the infected human stool. A suspension of 10⁶ cysts/0.2 ml in normal saline was administered orally to each rat. A group of 4-6 rats was sacrificed subsequently on 7, 11, 15, 22, 30 and 42 day of post-infection. The control rats for each group received 0.2 ml of normal saline. Animals were sacrificed under light ether anaesthesia. After flushing the intestine with 1 ml of chilled normal saline, the trophozoites were counted in the drained fluid. A portion of upper jejunum was immediately removed and stored in liquid nitrogen for RNA isolation. Remaining part of the intestine was used for various biochemical studies.

**Glucose uptake measurement**

D-Glucose uptake was determined in the presence and absence of Na⁺ as described. Everted intestinal segments (0.3-0.5 cm) were incubated at 37°C for 5 min in oxygenated buffer [Tris-maleate, pH 7.2, 120 mM NaCl or choline chloride (Na⁺-free buffer), 0.6 mM CaCl₂ and 1.21 mM MgSO₄, osmolarity was maintained to 300 mosmole] containing 5 mM D-glucose and trace amounts of [U-¹⁴C] D-glucose. Sugar uptake was measured by determining the

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*Author for correspondence*
overnight. The membrane was next washed twice was hybridized with [γ-32P]-labelled probe at 42 °C. Next, the labelled probe was added and membrane was hybridized with [γ-32P]-labelled probe at 42 °C overnight. The membrane was next washed twice with 2 × SSC/0.1% SDS solution and finally with 2 × SSC at room temperature. The membrane was exposed to X-ray film for autoradiography at -80 °C for at least 24 hr. The probe for β-actin used as an internal standard was obtained from Boehringer Mannheim.

Results

As shown in Table 1. *G. lamblia* trophozoites count in infected rat intestine was significantly higher at day 7 to 15 compared to that in the control. The count was highest at day 11 post infection (16080±104), although the infection was established as early as day 4 after exposure to the parasite. There was a gradual decline in the number of trophozoites in the gut after day 15 and it was equal to control levels by day 42 post-infection. As expected intestinal uptake of glucose in infected animals was significantly low compared to the control (Table 2). The uptake rate was the least (8.96±1.33 μ moles/5 min/g tissue) at day 11 when the degree of parasitemia was the

<table>
<thead>
<tr>
<th>Post infection (days)</th>
<th>Trophozoites (per ml)</th>
<th>D-Glucose uptake (μ moles/S min/g tissue)</th>
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<tbody>
<tr>
<td>Control</td>
<td>107±21</td>
<td>18.12±1.41</td>
</tr>
<tr>
<td>7</td>
<td>4620±73</td>
<td>10.09±1.26**</td>
</tr>
<tr>
<td>11</td>
<td>16080±104</td>
<td>8.96±1.33**</td>
</tr>
<tr>
<td>15</td>
<td>1391±164</td>
<td>9.73±1.67**</td>
</tr>
<tr>
<td>22</td>
<td>1986±38</td>
<td>13.13±3.42*</td>
</tr>
<tr>
<td>30</td>
<td>560±28</td>
<td>14.7±1.92</td>
</tr>
<tr>
<td>42</td>
<td>1982±20</td>
<td>16.56±2.26</td>
</tr>
</tbody>
</table>

* p<0.01 vs control; ** p<0.001 vs control

<table>
<thead>
<tr>
<th>Group</th>
<th>Kᵢ (mM)</th>
<th>Vₘ₉₅ (μ moles/5 min/g tissue)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>4.34±0.85</td>
<td>29.8±2.42</td>
</tr>
<tr>
<td><em>G. lamblia</em> infected (11 day)</td>
<td>4.34±0.93</td>
<td>13.2±1.96*</td>
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*p<0.001 vs control
highest. Glucose uptake was almost restored to control levels by day 22 post-infection. There was no change in glucose uptake in Na⁺ free medium in the control and infected animals under these conditions. Kinetic studies revealed, that the observed decline in the absorption of sugar in infected intestine was a consequence of decrease in \( V_{\text{max}} \) from 29.3±2.4 in the controls to 13.2±1.9 μ moles/5 min/g tissue in infected animals. There was no change in the value of apparent \( K_t \) (4.34 mM) under these conditions (Table 3).

**Effect on brush border disaccharidases**

The brush border sucrase and lactase activities exhibited a gradual decline with the progression of infection compared to the control (Table 3). The enzyme levels were least at day 11 post infection compared to that in control. The values were 0.627±0.116 for sucrase and 0.039±0.007 for lactase, as compared to the control group 0.982±0.103 and 0.055±0.016 units/mg protein respectively. However, as the degree of infection declined, the activities of both sucrase and lactase were found restored to control levels.

**mRNA levels of SGLT1 and brush border disaccharidases**

In order to understand how the uptake of Na⁺-dependent D-glucose and activities of disaccharidases declined in giardia infection, levels of mRNA encoding these proteins were examined by Northern blot analysis. As shown in Fig. 1a, Na⁺-glucose co-transporter oligonucleotide probe hybridized with 4.5 kb and 2.3 kb mRNA transcripts in the rat jejunum of infected as well as of the control. The densitometric scan of the data is shown in Fig. 1b. However, the intensities of the bands were markedly different on

<table>
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<th>Table 3—Effect of G. lamblia Infection on brush border disaccharidases (μ moles substrate hydrolyzed/min/mg protein)</th>
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<tr>
<td>[Values are mean ± SD of 4-6 animals in each group]</td>
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<tr>
<td>Post-infection (days)</td>
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<tr>
<td>Control</td>
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<tr>
<td>7</td>
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<td>11</td>
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*\( p<0.01 \) vs control; **\( p<0.001 \) vs control

![Fig. 1](image-url)

(a) Northern blot analysis of jejunal SGLT1 mRNA levels in control and G. lamblia infected rats [Total RNA/lane (10 μg) was resolved on 1% agarose-formaldehyde gel electrophoresis, transferred to Nylon membrane hybridized with \( \gamma^3P \) labelled oligonucleotide probe for human intestinal SGLT1 and autoradiographed. Bands show 4.5 kb fragment (top) and 2.8 kb fragment (bottom). Lane 1, control; lanes 2, 3 and 4, day 7, 11, and 15 post-infection, when infection was at the peak; lanes 5, 6, and 7, day 22, 30 and 42 post-infection respectively, when infection was on decline]; (b) densitometric scan of the blot for 4.5 kb SGLT1 fragment; (c) Northern blot analysis of jejunal epithelial β-actin mRNA in control and giardia infected groups respectively. The mRNA blot was hybridized with \( \gamma^3P \) β-actin probe]
each day post-infection compared to the pre-infected controls. Northern blot analysis revealed that mRNA at day 7 post-infection showed faint signals compared to that in the control. The mRNA signals on 11 and 15th day post-infection were essentially invisible as the degree of infection receded, the two mRNA bands (4.5 kb and 2.3 kb) gradually reappeared after day 15. On day 22 to 42 post infection, 4.5 kb and 2.3 kb mRNA fragments were fully restored to the control levels. Northern blot for sucrase and lactase in infected animals also revealed low mRNA levels at day 11 post-infection compared to the control. The mRNA signal corresponding to 6.5 kb and 6.8 kb for sucrase and lactase respectively were restored to almost control values by day 22 post-infection (Fig. 2a, 3a, 2b and 3b). There was no change in mRNA signal hybridized with β-actin probe used as an internal standard under these conditions (Fig. 1c).

Discussion

Among the many theories proposed to explain the intestinal functions in giardiasis are: (i), mechanical obstruction of microvillar surface due to the abundance of trophozoites in the gut which may impair the absorption of nutrients; (ii), mucosal damage to the small intestine; (iii), an increase, in the turn over rate of enterocytes across crypt villus axis such that the presence of large proportion of immature cells on the villus tip may retard the normal digestive and absorptive functions; (iv), the bacterial overgrowth may occur resulting in the formation of a barrier over the microvillus surface; (v), an increase in the secretion of mucus or mucoid substances may also be responsible for the observed malabsorption and

![Fig. 2—Northern blot analysis of jejunal sucrase mRNA (6.5 kb fragment) expression in G. lamblia infected rats (a); Lane 1, control group; lanes 2-4, day 7, 11 and 15 post-infection respectively, when infection was highest; lanes 5-7, day 22, 30 and 42 of post-infection when infection gradually declined; (b), densitometric scan of the data]

![Fig. 3—Northern blot analysis of jejunal lactase mRNA (6.8 kb fragment) expression (a); Lane 1 control; and lanes 2-7, G. lamblia infected, day 7, 11, 15, 22, 30 and 42 post-infection in rats; (b), densitometric scan of the blot]
(vi), an increase in gut motility in giardiasis. The observed decrease in Na\(^+\)-dependent D-glucose uptake and in the activities of sucrose and lactase in infected animals at day 11 post-infection are in agreement to the previous reports. Kinetic studies revealed that the decrease in glucose uptake was a consequence of reduced \( V_{\text{max}} \) with no change in the apparent \( K_{\text{m}} \) in infected rats. This indicates a decrease in the number of transporter molecules in infected enterocytes. Interestingly, there was no change in Na\(^+\)-independent glucose uptake under these conditions. It may be inferred that the giardial infection primarily disrupts the Na\(^+\)-dependent sugar uptake system which is mainly located in the mucosal side of the epithelium in rat intestine.

Northern blot analysis showed a considerable decrease in the level of mRNAs encoding Na\(^+\)-glucose co-transporter, sucrase and lactase activities at the peak of infection (day 11 post infection). Possibly there is down regulation of the Na\(^+\)-glucose co-transporter and brush border disaccharidases in *G. lamblia* infected rat intestine. However, the level of mRNA encoding these proteins were restored to almost control levels by day 22 post infection. Mac Donald et al. reported an increase in the turnover rate of enterocytes across crypt villus axis in *G. lamblia* infected small bowel and this may result in a large population of immature enterocytes having low levels of the transporter and brush border disaccharidases. Under such conditions, a general decrease in other brush border enzymes would be expected. However, no such changes have been observed in the activities of leucine amino peptidase, or \( \gamma \)-glutamyl transpeptidase in infected animals (unpublished results). These enzymes together with sucrose and lactase constitute ectoenzymes which are peri-pherally embedded in the microvillus membrane. Disruption of microvilli in *G. lamblia* infected intestine has been reported. However, Anand et al. did not find any change in the cytoplasmic enzymes of the epithelial cells in *Giardia* infected rats. This suggests that the enterocytes remained intact under these conditions. The oligonucleotide probes hybridized with 4.5 kb mRNA fragment which matches the size of mRNA coding for Na\(^+\)-glucose co-transporter in rat intestine. Besides, the oligonucleotide probe also weakly hybridized with another mRNA transcript of 2.3 kb size. Coady et al., have reported homology between cDNA clones of human and several animal species including rat for the intestinal Na\(^+\)-glucose cotransporter. cDNA probe employed by these workers was shown to hybridize strongly with 4.5 kb and weakly with 2.3 kb transcripts in rat intestine. It has been suggested that some other proteins present may contain the same coding region with different length of 3' untranslated region of the mRNA. In the present study, 2.3 kb mRNA fragment almost disappeared on day 11 and 15 post infected rats, while 4.5 kb transcript signal was faintly visible in these animals. This is consistent with the findings of Coady et al. that 4.5 kb mRNA transcript mainly codes for Na\(^+\)-glucose co-transporter in rat intestine. Oligonucleotide probes for sucrase and lactase hybridized with 6.5 kb and 6.8 kb transcripts for sucrase and lactase respectively which is in agreement with the size of mRNA reported by Kwo-yih et al.

A better northern blot for lactase could not be obtained even after repeated efforts. It is presumably due to low enzyme activity in adult rat intestine. However, it followed the same pattern of mRNA expression as Na\(^+\)/glucose co-transporter under these conditions.

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