Folate Deficiency Results in Alteration in Intestinal Brush Border Membrane Composition and Enzyme Activities in Weanling Rats

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Summary Folic acid deficiency is the most prevalent vitamin deficiency throughout the world and its effect on brush border membrane composition has not been studied earlier. We investigated the effect of folate deficiency on the structure and function of the intestinal brush border membrane. Various brush border enzyme activities, membrane sugars and lipids were evaluated in two groups of weanling male albino rats after 3 mo of feeding control and folate deficient diets. Except sucrase, all the other three enzymes, viz., alkaline phosphatase, leucine amino peptidase and γ-glutamyl transeptidase showed decrease in activity in rats fed folate-deficient diets. Among sugars, hexoses and hexosamines showed significant decline in amount whereas sialic acid content showed great increase in brush border membrane of folate-deficient rats as compared to controls. Furthermore, there was a significant reduction in cholesterol, phospholipids, triglycerides, cerebrosides and fucolipids in the group fed the folate-deficient diet. Our study suggests that folate deficiency results in altered enzyme activities, lipid and sugar composition of intestinal brush border membrane. Such changes might reflect the underlying cause of the gastrointestinal disturbances observed in folate deficiency.

Key Words folate deficiency, brush border membrane, lipid composition, brush border enzymes

Folate, a water soluble vitamin, is a member of vitamin B group and provides the one-carbon units for purine, thymidylate and amino acid synthesis and for methylation of a wide variety of biological molecules (1). As mammals are unable to synthesize folate de novo, it must be obtained either from the diet or by microbial breakdown in the gut. Although folates are well distributed in foods, they are susceptible to many physico chemical stresses that decrease their availability. In addition, an increased requirement or interference with folate metabolism by drugs can lead to folate deficiency, as observed in elderly people, pregnant women, smokers, alcoholics and contraceptive users (2). Folate deficiency is the most prevalent vitamin deficiency throughout the world with a significant number of cases resulting from the impairment of intestinal absorption (3). Severe folate deficiency can cause megaloblastic anaemia while low folate intake during pregnancy is associated with neural tube defects (4). In addition folate deficiency is known to complicate several gastrointestinal illnesses in which the risk of developing intestinal malignancies is increased (5).

The intestinal tissue is among the most rapidly replicating tissues in the body; the immature cells in the crypt base divide and progressively migrate to the villus tip and acquire the characteristics of the mature enterocytes. The turnover time at the crypt base is 12–24 h and the migration time across the crypt-villus axis is 48–72 h (6). Folic acid plays an important role in DNA synthesis and its deficiency might result in aberrant regeneration of the rapidly turning over intestinal epithelium. Hermos et al. (7) reported enlarged nuclei, reduced mitosis of crypt cells, increased cellularity of lamina propria and shortened villi in intestine of folate-deficient animals. The brush border membrane, lining the luminal border of the epithelial cells, comprises various digestive enzymes and transport carriers intercalated in the lipid bilayer (8). Chemically, the membrane is composed of approximately 60% proteins, 30% lipids and 10% carbohydrates (9). The effect of folate deficiency on the composition and function of the intestinal brush border membrane has not been known; hence the present study was designed to study the effect of folate deficiency on the brush border membrane composition and enzyme activities in the rat intestine.

MATERIALS AND METHODS

Chemicals. Folic acid casei medium was purchased from HiMedia (India). N-Acetyl neuraminic acid, fucose, p-nitro phenyl phosphate and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, USA).

Animals. Weanling male albino rats (Wistar strain) weighing 40–50 g were obtained from the central animal house of the institute. These were housed in

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polypropylene cages in the departmental animal house under hygienic conditions at controlled temperature (23 ± 1°C) and humidity (44–55%). The rats were randomized into two groups of four animals each. Folate deficiency was produced in a group of rats by feeding them a folate-deficient diet and 10 g succinylsulfathiazole per kg diet for 3 mo. Inclusion of succinylsulfathiazole facilitates induction of severe folate deficiency because this antibiotic eradicates intestinal microflora. Earlier studies have shown that dietary succinylsulfathiazole also inhibits other vitamins synthesized by intestinal microflora, mainly vitamin B_{12} and vitamin K. As this antibiotic was not included in the diets of control rats, the observed effects under these conditions can not be solely attributed to folate deficiency. However, this was taken care of, at least in part, by substitution of the recommended amounts of all other vitamins except folate in the diets of folate-deficient animals.

RESULTS AND DISCUSSION

The present study was undertaken to investigate the effect of folate deficiency on the intestinal brush border enzymes, membrane sugars and lipids. Folate deficiency was created by omitting folate and adding succinylsulfathiazole to the diet. This non-absorbable antibiotic drug inhibits the de novo synthesis of folate by the intestinal microflora of the gut. Earlier studies have shown that dietary succinylsulfathiazole did not affect the intestinal function as determined by fat and xylose absorption (25) and thiamine absorption (26). Succinylsulfathiazole also inhibits other vitamins synthesized by intestinal microflora, mainly vitamin B_{12} and vitamin K. As this antibiotic was not included in the diets of control rats, the observed effects under these conditions can not be solely attributed to folate deficiency. However, this was taken care of, at least in part, by substitution of the recommended amounts of all other vitamins except folate in the diets of folate-deficient animals.

Figure 1 shows the growth curves of rats from the control and folate-deficient groups during the period of the experiment. Consistent with the previous studies (27, 28), the folate-deficient rats showed progressive growth retardation beginning at week 4 of the dietary

Table 1. Composition of diet.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>(g/kg diet)</th>
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<tbody>
<tr>
<td>Casein</td>
<td>180</td>
</tr>
<tr>
<td>Starch</td>
<td>265</td>
</tr>
<tr>
<td>Sucrose</td>
<td>265</td>
</tr>
<tr>
<td>Cellulose</td>
<td>110</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>60</td>
</tr>
<tr>
<td>Choline</td>
<td>2</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>1 mL</td>
</tr>
<tr>
<td>Folic acid</td>
<td>2 mg</td>
</tr>
<tr>
<td>Corn oil</td>
<td>110 mL</td>
</tr>
</tbody>
</table>

In the folate-deficient diet, folic acid was omitted and 10 g/kg diet succinyl-sulfathiazole was added.
and fola te-deficient rats were 234
intervention. The mean body weights of the control
membranes isolated from control and folate-deficient
analysis of various enzymes in purified brush border
rats, expressed on a dry-weight basis. Results of the
brush border membranes in control or folate-deficient
indicated a severe folate-deficient status for the folate-
weight (4.11 vs. 4.27
different between control and folate-deficient rats
levels in the control and folate-deficient
diet as compared to controls. At the time of sacrifice, the
levels at 4, 8 and 12 wk of feeding the folate-deficient
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serum folate levels in the control and folate-deficient
groups were 102
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this vitamin has earlier been shown to delay the maturation of epithelial
cells across the crypt villus axis (31, 32). Reduced brush border enzymes might be one of the underlying
causes of gut-associated clinical symptoms like gastrointestional disorders in folate-deficient rats.
Table 3 depicts the effect of folate deficiency on the
membrane-bound sugar content. It is evident from the
data there was a significant reduction in total hexose
(18%) and leucine aminopeptidase (12%) in folate-deficient rats as compared to controls. There was a significant
decrease in the activity of γ-glutamyl transpeptidase (p < 0.05) under these conditions. Earlier reports (29, 30) have also shown a generalized depression of intestinal brush border enzymes in folate-deficient rats. This might be due to a decreased number of mature villus cells as folic acid plays a crucial role in cell replication and proliferation and deficiency of this vitamin has earlier been shown to delay the maturation of epithelial cells across the crypt villus axis (31, 32). Reduced brush border enzymes might be one of the underlying
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4 aberrations of lipid metabolism in the liver and brain of methylation was observed to be depressed in the liver increased degradation of lipids. In an earlier study (cient rats could be due either to reduced synthesis or to fractions in the brush border membrane of folate-defi-

-ation might influence the expression of various receptors which are specifically recognized by viruses, bacteria or protozoa. It might also result in altered ecology of microflora in the intestinal lumen.

Further, analysis of lipid composition in brush border membrane lipids showed that folate deficiency led to a decrease in membrane lipids. There was a significant reduction in cholesterol ($p<0.05$), phospholipids ($p<0.001$), triglycerides ($p<0.01$), cerebrosides ($p<0.01$) and fuscolipids ($p<0.05$). There was decrease in gangliosides but the change was not statistically significant (Table 4). The drastic reduction of various lipid fractions in the brush border membrane of folate-deficient rats could be due either to reduced synthesis or to increased degradation of lipids. In an earlier study ($34$), aberrations of lipid metabolism in the liver and brain of folate-deficient rats were observed. Phospholipid $N$-methylation was observed to be depressed in the liver of folate deficient rats due to the depressed level of $S$-adenosyl methionine. The cholesterol/phospholipid molar ratio in the brush border membrane of folate-deficient rats was $1.6$ as compared to $0.8$ in controls. This suggests a decrease in the fluidity of the membrane in folate-deficient animals. Altered fluidity and lipid composition of membrane in response to folate deficiency might alter membrane permeability and integrity and hence its functions. The intestinal brush border membrane serves as a protective barrier against a variety of antigenic, mutagenic and toxic compounds, and the altered permeability might affect its barrier functions, too.

In conclusion, the present study suggests that folate deficiency results in altered enzyme activities and the lipid and sugar composition of intestinal brush border membrane. These changes might affect the protective functions of this membrane.

REFERENCES

3) Lashner BA, Provencher KS, Seidner DL, Knesebeck L, Brzezinski A. 1997. The effect of folic acid supplementation on the risk for cancer or displasia in ulcerative col-

Table 4. Effect of folate deficiency on brush border membrane lipid composition.

<table>
<thead>
<tr>
<th>Lipids</th>
<th>Control</th>
<th>Folate deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>181.8±10.6</td>
<td>163.6±2.9*</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>548.7±15.8</td>
<td>223.8±36.4***</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>43.0±2.5</td>
<td>34.0±2.6**</td>
</tr>
<tr>
<td>Gangliosides</td>
<td>0.75±0.3</td>
<td>0.45±0.2</td>
</tr>
<tr>
<td>Cerebrosides</td>
<td>208.9±47.8</td>
<td>90.2±8.3***</td>
</tr>
<tr>
<td>Fuscolipids</td>
<td>45.4±15.8</td>
<td>19.3±6.6*</td>
</tr>
</tbody>
</table>

Values (µg/ng protein) are mean±SD, n=4. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs. control.


