Glucose-galactose malabsorption: demonstration of specific jejunal brush border membrane defect

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SUMMARY Jejunal brush border glucose transport was studied in a patient with glucose-galactose malabsorption and in controls, using jejunal brush border membrane vesicles (BBMV) prepared from conventional jejunal biopsies. Whereas BBMV from controls showed a seven-fold enhancement of D-glucose uptake in the presence of an inwardly directed sodium gradient compared with its absence, no such enhancement was seen in the patient’s vesicles. In BBMV from the patient, initial D-glucose uptake under sodium gradient conditions was only 10% of the mean control value. In contrast, sodium/proton exchange in BBMV from the patient was intact. These data provide the first unequivocal evidence that the jejunal brush border membrane is the site of a specific defect in sodium dependant glucose transport in glucose-galactose malabsorption. Measurement of glucose uptake by BBMV may well be the optimal diagnostic technique in this disorder.

A congenital defect in the intestinal absorption of glucose and galactose was first described in 1962 in two babies with profuse neonatal diarrhoea. Over 30 cases have now been reported in the literature. The defect is specific to glucose and galactose absorption, and fructose absorption, jejunal mucosal morphology, and disaccharidase activities are all normal. Clinical tolerance to the offending monosaccharides may improve with age and some patients are able to tolerate some dietary glucose. Despite the relative rarity of the disorder, a number of different experimental techniques have already been used in an attempt to elucidate the precise nature of the transport defect. Indirect techniques, using perfusion studies, whole mucosal glucose uptake in vitro, and Ussing chamber studies, have all produced evidence which is consistent with a translocation defect in the small intestinal enterocyte. Direct examination of glucose transport at the presumed site of the defect, the brush border membrane, however, has not so far been undertaken.

A technique has recently been evolved for measuring human brush border glucose transport using brush border membrane vesicles (BBMV), prepared from conventional per oral jejunal biopsies, and we have used this to investigate a child with glucose-galactose malabsorption.

Methods

The suspected transport defect was investigated in two ways. First, by in vivo perfusion studies to show that glucose and galactose absorption was defective in the patient. Second, by in vitro studies of sugar transport across the patient’s jejunal brush border membrane, the suspected site of the defect.

CASE REPORT

The patient, a boy, was born at term weighing 3·6 kg. In the newborn period repeated episodes of profuse watery diarrhoea and severe hypernatraemic dehydration followed the administration of human milk. Fructose was found to be the only carbohydrate tolerated orally. A presumptive diagnosis of glucose-galactose malabsorption was made on the ninth day of life, and the patient has subsequently grown and

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Received for publication 21 June 1988.
developed normally on a fructose based diet. Jejunal perfusion studies were done at the age of five months, and the jejunal biopsy was obtained at the age of three years.

CONTROL SUBJECTS

Jejunal perfusion studies

Sixteen control subjects, aged between two and 11 months, were being investigated for suspected malabsorption with failure to thrive, but after extensive investigation were shown to have no organic gastrointestinal disease; the jejunal mucosal morphology was normal in each case.

Jejunal brush border membrane vesicle studies

Histologically normal jejunal biopsy specimens were obtained from two sources: (i) partial thickness specimens (epithelium plus submucosa), weighing 20–50 mg, obtained per orally from five patients (aged one to six years) being investigated for gastrointestinal symptoms, but subsequently shown to have no organic disease; (ii) full thickness specimens weighing 90–150 mg, obtained surgically from 10 patients (aged two to seven months), undergoing a portoenterostomy procedure for extrahepatic biliary atresia. The specimens were rinsed rapidly in 0.15 M NaCl solution and stored in air tight containers at −70°C. There were no differences in the transport characteristics of vesicles prepared from the two sources.

All studies were carried out with the approval of the local Research Ethical Committee and the written, informed consent of the patients’ parents.

PERFUSION STUDIES

Net rates of jejunal monosaccharide absorption and lactose hydrolysis (luminal disappearance of intact disaccharide) were measured using a steady state perfusion technique. The perfusates contained glucose, galactose, or fructose (200 mmol/l) in deionised water, and polyethylene glycol 4000 (1 g/100 ml). Solutions were perfused in random order at 1 ml/min over a 30 cm segment of proximal jejunum, and the effluent collected at 4°C.

Measurements of net sugar transport were made in the steady state, 120 minutes after the start of perfusion. Preliminary studies in control subjects, showed that under these perfusion conditions, with a relatively low flow rate, no differences were present in net sugar absorption between sugar solutions made up in either saline solution (0.9 g/100 ml), and those made up with water. Furthermore the electrolyte composition of the effluent using the two types of solution was not significantly different. The sugar concentration in the perfusate (200 mmol/l) was higher than that used in many other perfusion studies, but is equivalent to the lactose concentration in human milk. Furthermore, in studies using a lower sugar concentration (50 mmol/l) total absorption of sugar usually occurred in control subjects, rendering the effluent solution sugar free, thereby making comparison of rates of sugar transport impossible.

Polyethylene glycol 4000 was estimated according to the method of Hyden. Sugar concentrations were determined enzymatically using Boehringer, Mannheim (BCL) test kits and reagents. Glucose was measured by the hexokinase procedure (BCL 124338); galactose with galactose dehydrogenase, and fructose and lactose were measured as glucose (BCL 124273) after treatment with isomerase and beta-galactosidase (BCL 105031). Transport rates were calculated using previously described formulae.

JEJUNAL BRUSH BORDER MEMBRANE VESICLE STUDIES

Isolation of Vesicles

Vesicles were prepared from biopsies using a previously described technique. In the case of peroral jejunal biopsy specimens, 20–50 mg frozen mucosa were thawed in 200 μl mannitol buffer containing (in mmol/l), mannitol 300; EGTA 5, Tris/HCl 12, pH 7.1. The buffer plus mucosa was diluted six fold by volume with ice cold, deionised water and homogenised for one minute (Sorvall Mini-Omnimixer, speed 5).

When using full thickness jejunal specimens taken at operation, 100–150 mg gut were thawed in 200 μl mannitol buffer, diluted six fold with ice cold, deionised water, and vibrated for two minutes (Vibro Mischer El, Chemap AG). After removal of any intestinal muscle with forceps, the material was homogenised as above.

Magnesium chloride was added to the homogenate to a final concentration of 10 mmol/l, and after standing on ice for 40 minutes, the specimen was centrifuged for 15 minutes at 3000 g at 4°C. The supernatant (I) was removed and stored on ice.

The pellet was resuspended in 1 ml buffer (in mmol/l), mannitol 60, EGTA 1, Tris/HCl 2.4, pH 7.1 and homogenised with a glass Teflon tissue homogeniser. After addition of MgCl₂ (10 mmol/l) and standing on ice for 15 minutes, the homogenate was centrifuged for 15 minutes at 3000 g at 4°C.

The supernatant (II) was added to supernatant I and the combined supernatants centrifuged for 30 minutes at 27000 g at 4°C. The resulting pellet was resuspended in a small volume of membrane buffer (about 125 μl) by means of a syringe and 25 gauge needle.
The membrane suspension was equilibrated at room temperature and 4°C for 30 minutes each, before transport studies.

**ENZYME ASSAYS AND PROTEIN DETERMINATIONS**

Two microvillus marker enzymes were used to assess brush border membrane purification. Alkaline phosphatase (EC 3.1.3.1) activity was measured using a fluorimetric procedure with 0.1M methyl umbelliferyl dihydrogen phosphate as substrate in borate buffer, pH 8.8, containing 5 mmol/l MgCl₂ and 0.1% Triton X-100; sucrase (EC 3.2.1.48) activity was determined fluorimetrically, using 0.028 M sucrose as substrate. The glucose product was measured by glucose oxidase-peroxidase with homovanillic acid as fluorogen.

Protein measurements were made with the Coomassie blue dye binding method, using the Bio-Rad protein assay kit.

Both brush border marker enzymes were measured in whole tissue homogenates and in the final vesicle preparations. Specific enzyme activities were enhanced 15–20-fold in the vesicle preparations.

**TRANSPORT STUDIES**

As a method control for confirming the integrity of BBMV, intact Na⁺/H⁺ exchange was confirmed in a fraction of each vesicle preparation. This requires a demonstration that an outwardly directed proton gradient (pH in<pH out) enhances sodium uptake, when compared with uptake without such a gradient (pH in=pH out). Vesicles were therefore pre-loaded with a buffer containing (in mmol/l): mannitol 300, 2(N-morpholino)-ethane sulphonic acid (MES) Tris (pH 6.0) 50, K⁺ gluconate 20, 0.1 mg/ml valinomycin, and incubated in a medium containing (in mmol/l): mannitol 300, NaCl 0.1% (³²Na tracer), K⁺ gluconate 20, and either Hepes-Tris (pH 7.4) or MES-Tris 50 (pH 6.0).

To show the presence of Na⁺-stimulated glucose uptake, glucose uptake under inwardly directed Na⁺-gradient conditions (Na out=Na in), was compared with glucose uptake under non-gradient conditions (Na out=Na in). Brush border membrane vesicles were therefore preloaded with one of two buffers, containing either (in mmol/l): mannitol 300 and Hepes-Tris 50 (pH 7.4); or mannitol 100, Hepes-Tris 50 (pH 7.4) and NaCl 100, and incubated in a medium containing (mmol/l): mannitol 100, Hepes-Tris 50 (pH 7.4), NaCl 100, and D-glucose 0.1% (+¹⁴H-D-glucose tracer).

The uptake of ³²Na or ¹⁴H-D-glucose (Amersham International) by BBMV after 15 seconds and 90 minutes (glucose) or 15 seconds and 120 minutes (³²Na) was performed in duplicate.

Table  Net rates of jejunal monosaccharide transport and of lactose hydrolysis in control children and the patient

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Galactose</th>
<th>Fructose</th>
<th>Lactose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=16)</td>
<td>169 (19)</td>
<td>173 (21)</td>
<td>132 (26)</td>
<td>150 (19)</td>
</tr>
<tr>
<td>Patient</td>
<td>28</td>
<td>45</td>
<td>117</td>
<td>131</td>
</tr>
</tbody>
</table>

Rates of sugar absorption and lactose hydrolysis are given as μmol/min/30 cm jejunum. Control values are mean (SD). The patient’s values are the means of two separate perfusion studies.

Figure  D-glucose and Na⁺ uptake in brush border membrane vesicles from controls and in a patient with glucose-galactose malabsorption. (a) D-glucose uptakes in the presence and absence of an inwardly directed Na⁺ gradient. (b) Na⁺ uptake in the presence and absence of an outwardly directed H⁺ gradient. The incubation media and membrane buffers used are given in the text. The control values are shown as mean (SEM). The patient’s values are the means of determinations on two separate biopsy specimens, each performed in duplicate.
(sodium) incubations, was measured using a rapid filtration technique:\textsuperscript{a}; 74 kBq \textsuperscript{2}Na or 185 kBq \textsuperscript{1}H-D-glucose were used per time point.

Results

**JEJUNAL PERFUSION** (Table)

In the patient, jejunal absorption of glucose and galactose was markedly impaired with reductions to only 17\% and 26\% respectively of the control means. In contrast, fructose absorption and lactose hydrolysis (the rate of luminal disappearance of intact lactose) were both normal. These data, together with the typical clinical history, confirmed the diagnosis of glucose-galactose malabsorption.

**JEJUNAL BRUSH BORDER MEMBRANE STUDIES** (Figure)

The data from controls showed enhanced 15 seconds uptake of D-glucose and Na\textsuperscript{+} under inward Na\textsuperscript{+}, or outward H\textsuperscript{+} gradients respectively. Furthermore, under inward Na\textsuperscript{+} gradient conditions, D-glucose uptake showed a two-fold overshoot at 15 seconds compared with the equilibrium value at 90 minutes, consistent with a secondary active transport process.\textsuperscript{7} The observations in control BBMV are therefore consistent with previously described Na\textsuperscript{+}-dependant D-glucose transport and Na\textsuperscript{+}/H\textsuperscript{+} exchange in the human jejunum.\textsuperscript{a}

Vesicles from the patient's biopsies showed marked abnormalities in D-glucose uptake at 15 seconds. There was no enhancement of uptake under Na\textsuperscript{+}-gradient conditions, and Na\textsuperscript{+}-stimulated uptake was significantly reduced to only 10\% of the control mean, showing a severe defect in brush border Na\textsuperscript{+}-dependent D-glucose transport. Additionally, at 15 seconds, under non-Na\textsuperscript{+} gradient conditions (Na\textsuperscript{+} 100 mmol/l inside and out), the patient's vesicles showed a paradoxically higher apparent glucose uptake than controls uptake at the 90 minutes equilibrium did not differ from controls.

Na\textsuperscript{+} uptake in vesicles from the patient was normal under all conditions. Thus, an outwardly directed H\textsuperscript{+} gradient enhanced 15 seconds Na\textsuperscript{+} uptake six fold compared with no gradient, consistent with intact Na\textsuperscript{+}/H\textsuperscript{+} exchange.

Discussion

The patient's data show for the first time that the jejunal brush border membrane is indeed the site of a defect in Na\textsuperscript{+}-dependant D-glucose transport in glucose-galactose malabsorption. Sodium stimulated glucose transport was markedly reduced, but Na\textsuperscript{+}/H\textsuperscript{+} exchange intact.

The question of whether the defect may be the result of reduced numbers of transport sites or alternatively, to defective carrier function, remains unresolved. Subsequent kinetic analysis using similar miniaturised methods may explain not only the observation that oral glucose and galactose tolerance may increase with age in affected patients,\textsuperscript{a} but may also permit investigation of the possibility of two or more molecular variants of this disorder.\textsuperscript{18} Based upon more than one class of glucose carrier,\textsuperscript{19,20} The observation in BBMV from the patient, that equimolar Na\textsuperscript{+} in both the extra- and intra-vesicular spaces led to enhanced D-glucose uptake, remains unexplained and could not be investigated further using osmotic collapse techniques.

This study has shown the value of BBMV techniques in defining the transport defect in glucose-galactose malabsorption. The technique is the most specific so far designed for investigating this disorder, and will be invaluable in further defining the nature of the molecular defect in this intriguing disease.

The authors are grateful to the Central Birmingham Health Authority Endowment Fund and the Children's Hospital Centenary Research Fund for the provision of financial support.

References


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*Gut* 1988 29: 1661-1665
doi: 10.1136/gut.29.12.1661

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