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Intestinal permeability as a measure of small intestinal mucosal integrity: correlation with jejunal biopsy

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Summary

The permeability of the small intestine was measured and jejunal biopsy performed in 39 children with gastrointestinal disorders. Intestinal permeability was measured using orally administered mannitol and lactulose as probe molecules in an isotonic solution (274 mOs/l), and the results were expressed as the ratio of the urinary excretion of the two sugars over 5 h. Urine samples were analysed for mannitol and lactulose content by high performance liquid chromatography. Children with small intestinal mucosal damage, irrespective of the cause, had a significantly lower ($P <$ 0.001) mannitol excretion (mean recovery 1.21% of ingested dose) than those with a normal mucosa (mean recovery 5.3%), while lactulose excretion did not differ ($P > 0.05$). The mean value of the lactulose:mannitol urinary excretion ratios was significantly higher ($P < 0.001$) in subjects with an abnormal mucosa (0.98) compared to those with a normal mucosa (0.2). Using the mean plus two standard deviations of the normal musosa group to define the upper limit of normal, all lactulose: mannitol excretion ratios from the abnormal mucosa group were above this limit. The results of this study show that the sugar permeability test is a sensitive, non-invasive screening test for jejunal mucosal damage in children and shows good correlation with jejunal biopsy results.

Résumé

La perméabilité de l'intestin grêle était déterminée et un biopsie jéjunale était prise chez 39 enfants avec des troubles gastrointestinaux.

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La perméabilité intestinale était déterminée employant les sucre lactulose et mannitole administrés par voie buccale, comme les molécules dan une solution isotonique (274 mOs/l) et les résultats étaient exprimés comme une proportion de l'excrétion des molécules pendant cinq heures. Les prélèvements de l'urine étaient analysés pour le mannitole et le lactulose par la méthode de la chromatographique liquide. Les enfants avec une membrane muqueuse abîmée sans égard pour la cause avaient une excrétion significativement plus bas ($P < 0.001$) de l'excrétion de mannitole (1.21% de la dose prise) que ceux avec une membrane muqueuse normale (5.3%), pendant que leur excrétion du lactulose n'était pas différent ($P > 0.05$). Le moyen proportion de l'excrétion de lactulose:mannitole était significativement plus ($P < 0.001$) chez les malades ave une membrane muqueuse anormale (0.98), comparé a ceux avec une membrane muqueuse normale (0.02). Employant le moyen plus deux écart-types du groupe avec une membrane muqueuse normale pour déterminer la haute limite de la normalité, tous les proportions de l'excrétion de lactulose:mannitole du groupe avec une membrane muqueuse anormale étaient au-dessus de cette limite. Les résultats de cette étude montrent que cet examen, employant la perméabilité du sucre est un examen sensitif et non-blessant pour déterminer si la membrane muqueuse est abîmée chez les enfants, et montrent aussi une bonne corrélation aux résultats des biopsies jéjunales.

Introduction

It has been suggested that changes in intestinal permeability which occur in the damaged mucosa of the small intestine, may be important

in the pathophysiology of various gastro-intestinal diseases [1,2]. In mucosal damage from a variety of causes, intestinal permeability changes in two ways; there is an increased up-take of undigested or partially digested macro-molecules, and there is also a malabsorption of the smaller molecules. Recently, there has been considerable interest in the question of altered intestinal mucosal permeability and this has led to the use of the phenomenon as a screening method for small intestinal mucosal damage [3–5].

In clinical practice, studies on adults using two sugars of different molecular size have shown that in villous atrophy, there is a de-creased absorption of the smaller molecule and an increased absorption of the larger molecule [3,4]. There are very few studies in children [5–7] but these have also suggested similarly abnormal permeability in childhood enteropathies. However, these few studies need further confirmation. Studies in adults have usually used hypertonic solutions, by the addi-tion of non-interfering osmotic fillers such as glycerol and lactose to the test solution. In younger children, this practice produced un-wanted problems such as nausea, vomiting or diarrhoea as reported by Pearson et al. [5].

This report extends the evaluation of the sugar permeability test as a non-invasive in-vestigation in children with a variety of gastro-intestinal disorders.

Subjects and methods

Children investigated

All children attending the gastroenterology clinic at Birmingham Children's Hospital (U.K.) over a 6 month period, who had a jejunal biopsy, were also subjected to the sugar permeability test. Of the 39 children investi-gated, 20 were boys and 19 were girls. All were Caucasian and their ages ranged from 5 months to 13 years (mean, 6.5 years). Their diagnoses were as follows: coeliac disease (13); cows' milk intolerance (2); other food allergies (5); irritable bowel syndrome (5); Crohn's disease (2); persistent diarrhoea (6); and failure to thrive (6). Tests were carried out in all 39 children before commencing any treatment regime. Six of the 39 (cows' milk intolerance, 2;

coeliac disease, 4) had repeat tests during treatment, at 6 weeks and 3–6 months respec-tively, after the pretreatment test. The children with coeliac disease were placed on a gluten-free diet while those with cows' milk intoler-ance were advised to withdraw milk from their diet. The dietary assessment and supervision were carried out by the hospital dietitian.

Biopsy procedure

Jejunal biopsy was obtained with a double-port Crosby capsule for paediatric use, under radio-logical guidance, from the proximal jejunum just distal to the ligament of Trietz. The biopsy specimen was examined with a dissecting micro-scope and processed for conventional histo-logical diagnosis. The histology was classified independently by the hospital histopathologist as either abnormal with partial (PVA) or subtotal villous atrophy (STVA), or essen-tially normal with normal villus and crypt architecture.

Sugar permeability test

The test solution comprised 7.5 g lactulose and 1 g mannitol dissolved in 100 ml water, giving an osmolality of 274 mOs. The sugar test was performed mainly on an out-patient basis, in the ward under the supervision of F.O.A. and the ward sister.

After fasting (overnight for older children and 4 h for infants and young children under 3 years of age), subjects emptied their bladders to provide a baseline urine sample, which was saved. One hundred millilitres of the test sol-ution was given per m^2 body surface area to each child up to a maximum total volume of 100 ml and a minimum of 20 ml. The child then continued fasting for the next hour. All urine passed within 5 h of the sugar drink was collected, with the addition of 0.5 ml of 20 g/100 ml sodium azide preservative. The volume was measured and 50 ml stored at $-20°C$ until it was analysed. Urine was col-lected with urinary bags in children below 2 years of age, while the older children passed urine into the urinals. In the former group, adequate measures were taken to ensure no faecal contamination of urine collected.

Mannitol and lactulose assay

The urine samples were analysed for mannitol and lactulose content using high performance liquid chromatography (HPLC). Samples were prepared for analysis by thawing, mixing and centrifuging at 5°C to remove any urine deposit, followed by desalting with Amberlite MB-1, acetate form (BDH Ltd, Poole, U.K.). The volume of desalted urine that contained 2 µmol creatine was purified further by thin layer chromatography on foil-backed silica gel-G plates using Haldorsen's solvent system [8]. Areas of the urine separations corresponding to mannitol and lactulose standards, run in parallel, were excised and eluted with acetonitrile:water (v/v) and the eluent dried under vacuum.

The residue was dissolved in 200 µl acetonitrile:water just prior to measurement of mannitol and lactulose content. Mannitol and lactulose were measured by HPLC using either a 250 × 5 mm Spherisorb-5 amino column (Phase Separations Ltd, Queensferry, U.K.), or a 250 × 4 mm LiChrosorb amino (E. Merck, Darmstadt, FRG) eluted with acetonitrile:water (70:30, v/v) at 1.5 ml/min. Eluted solutes were detected with a mass detector (Applied Chromatography System, Macclesfield, U.K.) and quantification was achieved by comparison with standard mannitol and lactulose mixtures, chromatography peak areas being measured with a Milton-Roy C1-10 integrator (Stone, U.K.). Tests with urine from fasting subjects, containing more than a trace of solutes with retention times corresponding to mannitol or lactulose were discarded. All analyses were done in duplicate after the desalting stage and means were taken for calculation of lactulose:mannitol ratios. Recovery of mannitol and lactulose added to urine

was ≥85%. The coefficient of variation of differences between duplicate analyses was 2.5%.

Statistics

The significance of differences between group means was determined using Student's *t*-test.

Results

Forty-five biopsies and sugar tests (six repeats) were performed on the 39 subjects. The subjects were divided into two groups, the children with normal jejunal histology and the children with STVA or PVA.

Pretreatment tests

Table 1 shows the results of the urinary excretion of both sugars in the two groups of subjects. The subjects with normal histology excreted 1.56–19.72% (mean, 5.3 ± 4.14) of the ingested mannitol, while those with abnormal histology excreted significantly less of their mannitol load (mean, 1.21 ± 1.62; range, 0.26–7.0%). The difference between the means was highly significant ($P < 0.001$). The mean lactulose excretion of the normal histology group was 0.13% (range, 0.05–0.56%) less than the mean lactulose excretion for those with abnormal histology (0.35%; range, 0.019–4%). However, the difference was not significant ($P > 0.2$).

The excretion of the two sugars was expressed further as a ratio of lactulose (mg) to mannitol (mg). For those with normal histology, mean lactulose:mannitol ratio was 0.20 (range, 0.083–0.32), and for those with mucosal enteropathy was 0.98 (range, 0.50–2.23)

Table 1. Urinary recoveries of lactulose and mannitol in relationship to jejunal histology

Jejunal histology	Lactulose recovery (%)*	Mannitol recovery (%)*
Normal (*n* = 29)	0.13 ± 0.128	5.30 ± 4.14
Abnormal (*n* = 16)	0.35 ± 0.98	1.21 ± 1.62
t	0.894	4.707
P	>0.2	<0.001

*Mean ± standard deviation.

(Table 2). The difference was highly significant ($P < 0.001$). There was no overlap between the excretion ratios of the two groups (Fig. 1). The upper limit of normal for the lactulose:mannitol ratio (0.352) was defined as the mean plus two standard deviations ($\bar{x} + 2$ s.d.) of the ratios for those with normal histology.

Post-treatment tests

Six children who had repeat sugar tests and biopsies had had abnormal biopsies and high permeability ratios prior to treatment. Five of these children had a normal repeat biopsy and their lactulose:mannitol ratios fell from a clearly abnormal value to within the normal range (Fig. 2). The difference between

Table 2. Lactulose:mannitol ratios in relationship to jejunal histology

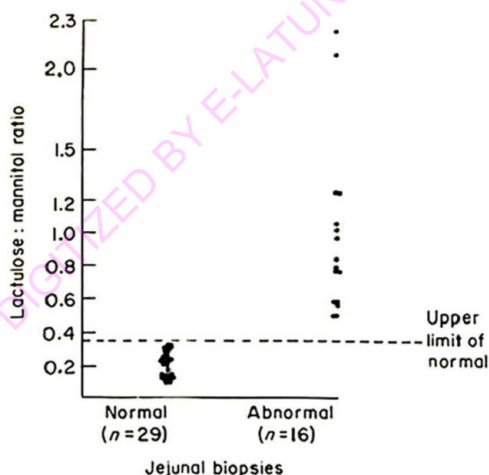| Jejunal histology	Lactulose:mannitol*
Normal ($n = 29$)	0.20 ± 0.076
Abnormal ($n = 16$)	0.98 ± 0.52
t	5.965
P	<0.001

*Mean $\pm$ standard deviation.

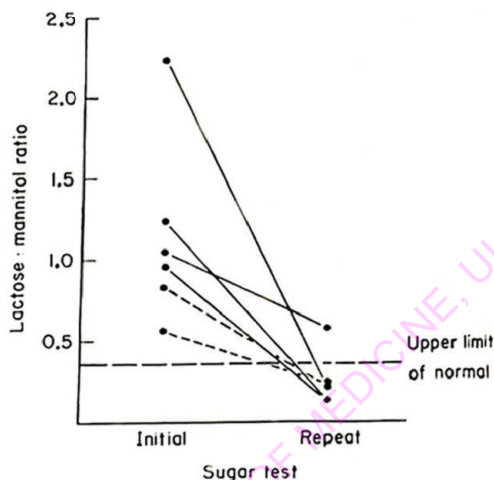**Fig. 1.** Lactulose:mannitol ratios in all subjects including the repeat tests.

Fig. 2. Lactulose:mannitol ratios in four coeliac subjects (———) and two subjects with intolerance to cows' milk (-------), before and after treatment.

the means ($\pm$ s.d.) of the ratios of the initial (1.16 ± 0.64) and repeat tests (0.18 ± 0.05) was significant ($P < 0.01$) (Table 3). Furthermore, their mean mannitol recovery rose significantly by sixfold from 0.7 to 4.27% ($P < 0.05$). Only one subject, a coeliac, with abnormal repeat biopsy, still had an abnormally high permeability ratio (Fig. 2).

Discussion

This prospective study confirms other reports which have attested to the usefulness of non-metabolizable dual sugar absorption tests as a non-invasive means of assessing mucosal damage in various gastrointestinal disorders in adults [3,4] and children [5–7]. It also confirms the report by Strobel *et al.* [9], that there is good correlation between the results of such tests and the histological findings in the jejunal mucosa. Mannitol and lactulose have been used as probe molecules in permeability tests, because of their physicochemical properties. They are hydrophilic and lipophobic and have no affinity for mediated transport. They are not metabolized, with almost total recovery in the urine after intravenous injection [1]. Their urinary excretion therefore reflects gastrointestinal absorption. The mechanism by which these sugars permeate through the epithelium

Table 3. Urinary recoveries of lactulose and mannitol and lactulose:mannitol ratios in five subjects who had repeat biopsies and sugar tests

Test	Lactulose recovery (%)*	Mannitol recovery (%)*	Lactulose:mannitol ratio*
Initial	0.12 ± 0.1 ($n = 5$)	0.7 ± 0.36 ($n = 5$)	1.16 ± 0.64 ($n = 5$)
Repeat	0.11 ± 0.09 ($n = 5$)	4.27 ± 3.08 ($n = 5$)	0.18 ± 0.05 ($n = 5$)
t	0.17	2.57	3.41
P	>0.5	<0.05	<0.01

*Mean ± standard deviation.

remains hypothetical. The permeability of the small intestine to water soluble substances, appears to be dependent upon molecular size. Since the small intestine is penetrated by pathways of radius 0.3–0.9 nm, with a higher population of the larger pathways being present in the jejunum than in the ileum [10], small molecules the size of mannitol (radius 0.4 nm; MW 182) may permeate these pathways with ease, while larger molecules like lactulose (radius 0.5 nm; MW 342) may not. However, lactulose is thought to pass through intercellular spaces where junctional complexes allow free passage of large molecules. Extrusion zones at villous tips may also play a part in the uptake of the disaccharide [3,4].

In this study, children with enteropathies, irrespective of the cause, absorbed significantly less mannitol than those with a normal mucosa, presumably as a consequence of the reduced surface area. Although the mean lactulose output by the children with an abnormal mucosa was higher than that for children with a normal mucosa, the difference was not statistically significant. This supports the findings of Pearson et al. [5] in children, but is at variance with findings from adult studies [3,4] which have shown an increased absorption of the disaccharide used. The lack of difference in the lactulose output by the two groups in this study may be due to the low osmolality of the oral solution compared to that used in adult studies. Menzies et al. [4] and Wheeler et al. [11] have shown that there is a correlation between osmolality and the amount of sugar absorbed.

The higher the osmolality of the solution, the more of the disaccharide is absorbed by the damaged mucosa, and for this reason, studies on adult populations have used hypertonic solution produced by addition of glycerol, lactose or sucrose to the test solutions.

Previous methods for measuring intestinal permeability involved the ingestion and urinary measurement of a single non-metabolized sugar, such as D-xylose. The results obtained using these single sugar methods are influenced by non-mucosal factors such as gastric emptying, intestinal transit, dilution by intestinal fluid, renal clearance and reliability of urine collection. The simultaneous ingestion of two sugars in the test solution, as in this study, conveniently avoids the effects of the variables mentioned above. Although these variables may alter the total recovery of the sugars in the urine, they make little difference to the urinary excretion ratio as the variables affect both components similarly, so that the ratio is determined solely by the state of intestinal permeability. Expression of the urinary recoveries of these sugars as a ratio has been shown to enhance further the discrimination of normal from abnormal mucosa, which the recovery of either marker alone fails to demonstrate clearly. The surprisingly high ratios obtained with abnormal histology, and confirmed in this study, are due principally to the significant reduction in the absorption of mannitol by the damaged mucosa. As pointed out by Hamilton et al. [12] and supported by the results of the six children who had repeat tests,

the sugar test may prove useful in following the response to treatment of patients with mucosal damage.

In developing countries where intestinal infections, infestations and malnutrition continuously damage the small bowel mucosa, the sugar test will be an invaluable screening test to determine the effects of the resulting mucosal morphological alteration on function. Although the permeability test is not diagnostic of any disease, it is a sensitive screening test for small intestinal enteropathy and thus of small intestinal function in various gastrointestinal conditions. An abnormal ratio, however, must be considered as an indication for the definitive procedure for demonstrating enteropathy, such as the performance of the jejunal biopsy.

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