Effects of pre- and post-absorptive factors on the lactulose/rhamnose gut permeability test

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ABSTRACT

It is assumed that the outcome of the lactulose/rhamnose gut permeability test is not influenced by pre- or post-absorptive factors. The aim of our study was to investigate the role of a pre-absorptive factor, i.e. small-intestinal transit, and a post-absorptive factor, i.e. renal clearance. Ten healthy male subjects were studied. Urinary lactulose and rhamnose excretion was measured after intraduodenal administration of lactulose and rhamnose following induction of increased intestinal permeability using chenodeoxycholic acid (chenodiol), in the absence and in the presence of accelerated intestinal transit. Urinary sugar excretion was measured after intravenous administration of either a regular dose (50 mg/50 mg) or a high dose (250 mg/250 mg) of lactulose/rhamnose. The intraduodenal experiments showed that a combination of accelerated small-bowel transit and increased permeability did not lead to significant differences in the recovery of lactulose ($P_{fl} = 0.647$) or rhamnose ($P_{fl} = 0.889$), or in the lactulose/rhamnose ratio, compared with those under conditions of increased permeability alone ($P = 0.68$). However, lactulose recovery was significantly lower ($P = 0.025$) after intravenous administration of a high dose of the sugars. There was no significant difference in urinary rhamnose recovery ($P = 0.575$) between the high and the regular doses. This resulted in a significantly lower lactulose/rhamnose ratio ($P = 0.021$) after intravenous administration of a high dose, compared with a regular dose, of the sugars. In conclusion, the assumption that post-absorptive processes do not influence the outcome of the lactulose/rhamnose permeability test appears not to be valid.

INTRODUCTION

Intestinal permeability can be evaluated by measuring the urinary excretion of orally administered water-soluble, non-degradable test molecules. This barrier function test is based on a comparison of the intestinal permeation of a larger molecule with that of a smaller molecule, by measuring the ratio of urinary excretion of these molecules. These two molecules follow different routes of intestinal permeation; the larger molecules are assumed to permeate paracellularly, whereas the smaller molecules are assumed to permeate transcellularly.

Pre-absorptive factors such as gastric emptying, dilution by secretion and small-bowel transit time (SBTT), and post-absorptive factors such as systemic distribution and renal clearance, are assumed to affect both molecules equally. Therefore the urinary excretion ratio is considered to be a measure of intestinal permeability [1–3].

Key words: intestinal permeability, lactulose/rhamnose ratio, post-absorptive factors, pre-absorptive factors.

Abbreviation: SBTT, small-bowel transit time.

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Recently we demonstrated that, under conditions of increased intestinal permeability, the outcome of the lactulose/rhamnose test is influenced by the dose of lactulose/rhamnose administered [4]. This suggests a possible involvement of pre- and/or post-absorptive factors in addition to the process of intestinal permeation if the permeability is increased, which will determine the outcome of the test. Lactulose, for instance, accelerates small-bowel transit [5], hence leading to a decreased duration of exposure of both molecules to the intestinal mucosal surface. The effect of the duration of small-bowel transit on the outcome of the lactulose/rhamnose test has not been studied previously. Renal clearance of the sugars has only been studied after intravenous administration of a single dose of either lactulose or rhamnose [6]. The lactulose/rhamnose ratio has not been studied after intravenous administration of the sugars.

Thus the aim of the present study was to investigate whether small-bowel transit, which determines the duration of exposure of lactulose and rhamnose to the intestinal mucosal surface, as well as the process of renal clearance of the sugars, affect the outcome of the lactulose/rhamnose permeability test. First we compared the urinary recovery of lactulose and rhamnose after intraduodenal administration of the sugars under control conditions, under conditions of increased permeability, which was induced artificially by oral administration of 750 mg of chenodeoxycholic acid (chenodiol) [7], and under conditions of both increased permeability and accelerated small-bowel transit, which was induced artificially by oral administration of 10 mg of cisapride, a prokinetic compound which does not influence intestinal permeability. Secondly, we compared the urinary lactulose/rhamnose ratios after intravenous administration of either a regular or a high dose of the sugars.

METHODS

Subjects
A group of 10 healthy, non-smoking male subjects (median age 25 years; range 18–29 years) were studied in the morning after an overnight fast. The subjects were not allowed to consume alcoholic beverages, to use drugs or to perform intensive physical exercise on the day prior to the testing days. The study was approved by the Ethics Committee of the University Hospital Maastricht. The research has been carried out in accordance with the Declaration of Helsinki. All subjects gave their written informed consent to participate in the study.

Design of the study
The subjects were studied on five occasions. First they underwent, at random, three small-bowel transit experiments. Secondly they underwent, at random, two renal clearance experiments.

Intestinal transit experiments

Small-bowel transit experiments

A charriere 12 Bengmark-type naso-intestinal catheter (Flocare, Zoetermeer, The Netherlands) was placed in the duodenum. The correct positioning of the catheter was confirmed using fluoroscopy. The subjects were studied in three separate experiments, carried out in a random order. (1) Control experiment: a standard test solution containing 5 g of lactulose (Centrafarm, Etten-Leur, The Netherlands) and 0.5 g of l-rhamnose (Sigma Chemical Co.) in 50 ml of saline solution was administered in 1 min. (2) Increased permeability experiment: the subjects received orally 750 mg of chenodeoxycholic acid (three 250 mg tablets; Chenofalk®; Falk Pharma Gmbh, Freiburg, Germany), which is known to increase intestinal permeability [6,7], 1 h before the administration of the standard test solution as in the control experiment. (3) Increased permeability and accelerated small-bowel transit experiment: the subjects received orally 750 mg of chenodeoxycholic acid plus 10 mg of cisapride (Prepulsid®; Janssen Cilag BV, Tilburg, The Netherlands) to accelerate small-bowel transit. At 1 h after administration of these compounds, the same solution as in the control experiment was administered.

In each experiment, total urine production was collected for 5 h after administration of the lactulose/rhamnose solution, its volume was measured and small portions were stored at −80 °C until analysis. All tests were separated by a washout period of at least 72 h. Urinary lactulose and rhamnose excretion was determined by a validated, sensitive, newly developed fluorescent detection HPLC system [8], and the lactulose/rhamnose ratio was calculated.

Measurement of SBTT

The solution that was administered intraduodenally contained 5 g of lactulose, which is a non-digestible soluble carbohydrate that allows the estimation of SBTT by measuring $H_2$ in exhaled breath. As soon as the lactulose enters the colon, bacterial fermentation takes place, and $H_2$ gas will be produced [9]. Every 15 min the subject breathed for 2 min through a mouthpiece, which was connected to a mixing chamber. Breath samples for $H_2$ analysis were collected from the mixing chamber at 15 min intervals, starting at $t = 0$, using a 140 ml syringe, and were analysed for $H_2$ enrichment using a sensitive electrochemical exhaled-hydrogen monitor (GMI Medical Ltd., Renfrew, Scotland, U.K.). SBTT was determined as the time that elapsed until the onset of a sustained increase in breath $H_2$, which was defined as the first breath sample which showed a higher level of $H_2$ than the
Intestinal permeability preceding one and which was followed by two or more breath samples showing a further increase.

Renal clearance experiments
A Teflon catheter was placed in an antecubital vein. Two different test solutions were administered on two separate test days in a random fashion. The test solutions, packaged in infusion bags, contained either 50 mg of lactulose and 50 mg of rhamnose in 100 ml of saline solution, or 250 mg of lactulose and 250 mg of rhamnose in 100 ml of saline solution. The infusion bags were prepared and examined in the Department of Clinical Pharmacy of the University Hospital Maastricht, to exclude microbiological and pyrogenic contamination. The test solutions were administered intravenously during a period of 60 min. Total urine production was collected for 5 h after the start of the infusion, its volume was measured and small portions were stored at −80 °C until analysis. All tests were separated by a washout period of at least 72 h.

Statistics
Data are presented as median (range). Comparisons between the intraduodenal experiments were carried out using Friedman’s and Wilcoxon’s non-parametric tests. Comparisons between the intravenous experiments were carried out using Wilcoxon’s non-parametric test. All statistical analyses were performed using the SPSS 7.5 for Windows package.

RESULTS
All subjects were able to complete all the tests without suffering from any ill effects.

Intestinal transit experiments
Small-bowel transit experiments
The results of the small-bowel transit experiments are displayed in Table 1. As expected, administration of chenodeoxycholic acid, either alone or in combination with cisapride, 1 h before ingestion of the lactulose/rhamnose solution resulted in a significantly increased permeability compared with the control experiment (control versus chenodeoxycholic acid, $P = 0.021$; control versus chenodeoxycholic acid + cisapride, $P = 0.022$). This led to significantly increased lactulose recovery ($P = 0.018$), while rhamnose recovery remained unchanged. Wilcoxon’s analysis demonstrated that administration of cisapride, leading to acceleration of small-bowel transit, did not result in a significant difference in lactulose recovery ($P = 0.647$), rhamnose recovery ($P = 0.889$) or lactulose/rhamnose ratio ($P = 0.68$) compared with administration of chenodeoxycholic acid alone.

SBTT
The results of the SBTT measurements are displayed in Figure 1. Friedman’s analyses showed a significant difference between the three experiments ($P = 0.009$). The SBTT values were as follows: control, 82.5 min (45.0–195.0 min); chenodeoxycholic acid, 90.0 min (45.0–150.0 min); chenodeoxycholic acid + cisapride, 60.0 min (30.0–165.0 min). Wilcoxon’s analysis demonstrated significantly accelerated small-bowel transit on administration of chenodeoxycholic acid + cisapride compared with chenodeoxycholic acid alone ($P = 0.047$). There were no significant differences in SBTT between control and chenodeoxycholic acid + cisapride or between control and chenodeoxycholic acid alone. No significant correlations were observed between individual sugar recoveries and SBTT.

Renal clearance experiments
The results of the renal clearance experiments are displayed in Table 2. Urinary lactulose recovery (%) was significantly lower if a high dose of the sugars was administered. However, there was no significant difference in urinary rhamnose recovery (%) between the high and regular doses. This resulted in a significantly lower lactulose/rhamnose ratio after intravenous

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Recovery (%)</th>
<th>Lactulose</th>
<th>Rhamnose</th>
<th>Lactulose/rhamnose ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.11 (0.05–0.72)</td>
<td>14.93 (4.83–30.48)</td>
<td>0.0101 (0.0026–0.0461)</td>
<td></td>
</tr>
<tr>
<td>Chen.</td>
<td>0.43 (0.13–0.84)</td>
<td>9.06 (2.98–13.04)</td>
<td>0.0438 (0.0241–0.1170)</td>
<td></td>
</tr>
<tr>
<td>Chen. + cisapride</td>
<td>0.27 (0.03–2.43)</td>
<td>8.93 (2.59–21.04)</td>
<td>0.0219 (0.0031–0.2241)</td>
<td></td>
</tr>
<tr>
<td>P-value (Friedman)</td>
<td>0.05*</td>
<td>0.65</td>
<td>0.021*</td>
<td></td>
</tr>
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</table>
administration of a high dose, compared with a regular dose, of the sugars.

**DISCUSSION**

In our recent study [4] we demonstrated that the dose–absorption kinetics of lactulose and rhamnose follow a non-linear function; if the permeability is increased, the lactulose/rhamnose ratio is higher if larger quantities of the molecules are applied. Although it is assumed that pre- and post-absorptive factors do not influence the outcome of the lactulose/rhamnose permeability test, the recovery data from the previous study [4] demonstrated that, if intestinal permeability is increased, the administration of a higher dose of lactulose and rhamnose results in unchanged lactulose recovery but in decreased rhamnose recovery. These data suggest a possible involvement of pre- and/or post-absorptive factors in addition to paracellular absorption if the permeability is increased, for instance lactulose-induced accelerated small-bowel transit [5]. In a recent study [10], lactulose and rhamnose were administered intravenously to rats both under normal conditions and under conditions whereby the animals were given fluid support. Their results demonstrated that urinary lactulose excretion increased dramatically if the animals received a fluid load, which suggests that renal clearance may also influence the outcome of the test.

In the present study we investigated the role of small-bowel transit by administering lactulose and rhamnose directly into the duodenum, thus avoiding possible disturbances due to gastric dilution and the process of gastric emptying. Small-bowel transit was accelerated using cisapride, a prokinetic compound which is not likely to interact with chenodeoxycholic acid and does not affect intestinal permeability. In this study we also investigated the combined role of systemic distribution and renal clearance of the sugars, by administering two different doses of the sugars intravenously, during a period of 1 h. The quantities of sugars administered (50 mg and 250 mg) were chosen arbitrarily, but both doses represent quantities that may occur in the circulation after ingestion of a lactulose/rhamnose solution. The period of 1 h during which the sugars were administered was also chosen arbitrarily to represent more or less the duration of exposure and permeation of lactulose and rhamnose to the small-intestinal mucosal surface. If renal clearance affects both molecules equally, as is assumed in the literature [1], it may be expected that administration of a low or a high dose of the sugars will not result in any change in the lactulose/rhamnose ratio.

The results of the present study demonstrated that acceleration of small-bowel transit from a transit time of 90 min to one of 60 min does not significantly influence the outcome of the test, as reflected by the lactulose/rhamnose ratio in the chenodeoxycholic acid experiment compared with that in the experiment in which both chenodeoxycholic acid and cisapride were administered. However, the possibility cannot be excluded that small-bowel transit influences the outcome of the lactulose/rhamnose test if the acceleration of small-bowel transit is more pronounced.

Our data showed lower lactulose recoveries if a higher quantity of lactulose was administered, while rhamnose recovery remained unchanged, which demonstrates that the process of renal clearance does not affect both molecules equally under all conditions. This is reflected by a higher lactulose/rhamnose ratio after intravenous administration of a low dose of the sugars. This ob-

<table>
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<th>Recovery (%)</th>
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<tr>
<td>Dose</td>
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<tr>
<td>Regular (50/100 mg)</td>
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<tr>
<td>High (250/250 mg)</td>
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<td>*P-value</td>
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Intestinal permeability suggests that the process of renal clearance is different for the two sugars. Data available in the literature regarding the recoveries of the sugars are not entirely equivocal. Maxton et al. [6] observed a urinary lactulose recovery of 75% and a rhamnose recovery of 62% at 5 h after an intravenous bolus injection of 500 mg of lactulose and 500 mg of rhamnose, which are very large quantities. In our study we observed similar rhamnose recoveries, but much lower lactulose recoveries. This difference may be explained, at least partially, by the different administration regimens of the sugars (a 10 ml bolus injection [6] compared with a 100 ml infusion for 1 h), and by difference in analytical techniques (estimation by TLC [6] compared with measurement using HPLC).

Summarizing, we provide evidence that a moderate acceleration of small-bowel transit does not influence the outcome of the lactulose/rhamnose permeability test. Since gastric emptying of liquids is normally a rapid process, one may conclude that the processes preceding intestinal uptake of the sugars do not influence the outcome of the lactulose/rhamnose permeability test. Renal clearance of rhamnose is not dependent on the quantity of rhamnose present in the circulation. Although there are no data available concerning the renal clearance of lactulose, it appeared that this clearance is dependent on the quantity of lactulose present in the circulation, as a higher quantity of lactulose resulted in a lower recovery at 5 h and a lower lactulose/rhamnose ratio, thus under-estimating a possible increase in permeability if more lactulose and rhamnose are absorbed by the small intestine. Hence the assumption that post-absorptive factors do not influence the outcome of the lactulose/rhamnose permeability test appears not to be valid, which may be of consequence for the usefulness and reliability of the test in patients.

REFERENCES