Kinetics of urinary recovery of five sugars after orogastric administration in healthy dogs

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Objective—To describe the kinetics of urinary recovery (UR) of 5 sugars used for gastrointestinal permeability and mucosal function testing following orogastric administration of lactose, rhamnose, xylose, methylglucose, and sucrose.

Animals—7 healthy male Beagles.

Procedures—A sugar solution containing lactulose, rhamnose, xylose, methylglucose, and sucrose was administered by orogastric intubation to healthy dogs. Urine samples were collected immediately before sugar solution administration (baseline) and at 2-hour intervals thereafter. The UR of the 5 sugars was determined from urine concentrations measured by high pressure liquid chromatography and pulsed amperometric detection. Percent urinary recovery (%UR) of the total UR up to 12 hours after sugar solution administration was calculated for each sugar at 2-hour intervals.

Results—Mean %UR exceeded 85% for all 5 sugars at 6 hours after orogastric administration of the sugar solution and exceeded 90% after 8 hours.

Conclusions and Clinical Relevance—In healthy dogs, a urine collection period of 6 hours is sufficient for gastrointestinal permeability and mucosal function testing following orogastric administration of lactulose, rhamnose, xylose, methylglucose, and sucrose. (*Am J Vet Res* 2002;63:845–848)

Gastrointestinal permeability and mucosal function testing in humans has been performed since the 1930s. Early investigators used rhamnose, xylose, and arabinose as marker molecules.[1] Several hundreds of scientific papers regarding gastrointestinal permeability and mucosal function testing in human and veterinary patients have been published. Many marker molecules have been used, and protocols for performing the tests and assay methods have been described.[2-7] However, until recently commercial quantification of marker molecules in urine was not routinely available, and no universally accepted protocol for gastrointestinal permeability and mucosal function testing had been described.[8] We have recently reported on the validation of a method for concurrent quantification of lactulose, rhamnose, xylose, methylglucose, and sucrose in urine of healthy dogs.[9]

The principle of gastrointestinal permeability and mucosal function testing is simple. A marker molecule or a mixture of marker molecules is administered orally or by orogastric intubation. The appearance of the marker molecule is then monitored in serum or urine. For most marker molecules the quantification is much more difficult in serum than in urine, and most studies[10,11] have reported quantification of the marker molecule in urine. Many marker molecules have been used, including monosaccharides and disaccharides, polyethylene glycol, and ^{51}Cr-EDTA.[12-15] Lactulose and rhamnose are used as markers of small intestinal permeability, whereas xylose and methylglucose are used as markers for small intestinal mucosal absorptive capacity.[10] Sucrose can be used as a marker for gastric permeability.[16] One important variable is the period for urine collection. Some investigators that have used monosaccharides and disaccharides for gastrointestinal permeability and mucosal function testing suggest that urine should be collected for a 5-hour period after administration of the sugar solution.[10,15,17] However, to identify an optimal period for urine collection, the study of the kinetics of **urinary recovery (UR)** is necessary. One group of investigators reported the kinetics of UR of several sugars after IV administration to healthy dogs.[18] However, to our knowledge the kinetics of UR of lactulose, rhamnose, xylose, methylglucose, and sucrose after orogastric administration in dogs have not been reported previously. Therefore, the purpose of the study reported here was to describe the kinetics of UR of 5 sugars used for gastrointestinal permeability and mucosal function testing following orogastric administration in healthy dogs.

Materials and Methods

Seven healthy dogs belonging to a research colony and owned by and housed at the Hill's Pet Care Center in Topeka, Kan, were entered into our study. All 7 dogs were male Beagles, 2 of which were sexually intact and 5 of which were castrated. Two of the dogs were 6 years old, 3 were 8 years old, and the remaining 2 dogs were 9 years old. Body weights ranged from 14.3 to 18.7 kg (mean ± SD, 15.8 ± 1.5 kg). Dogs were closely monitored on a daily basis, and none of them had any history of clinical signs suggesting gastrointestinal tract disease. Overall health of the dogs was also assessed by CBC determination and serum biochemical analysis. Blood samples were collected from the dogs approximately 6 months prior to our study and again 6 weeks after completion of our study. Laboratory test results before and after our study did not reveal any noteworthy abnormalities in any dogs. Physical examination did not reveal abnormalities in any of the 7 dogs.

Immediately before the beginning of the study, the urinary bladder of each dog was catheterized by use of a Foley urinary catheter and was completely emptied. An aliquot of 10 ml of urine was saved, and 10 µl of a NaN_3 solution (0.1 g/ml) was added as an antimicrobial agent. Samples were frozen at –70 C. Each dog was given 100 ml of a sugar solution containing 2.5 g lactulose,[a] 2.5 g L-rhamnose[b] (rham-

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nose), 2.5 g D (+) xylose[c] (xylose), 1.0 g of 3-O-methyl-D-glucopyranose[d] (methylglucose), and 10 g of sucrose[e] (sucrose) by orogastric intubation. Sugars were dissolved in tap water, resulting in a solution with an osmolality of approximately 745 mOsm/kg. The urinary bladder of each dog was catheterized with a urinary catheter and completely emptied at 2-hour intervals up to 12 hours after administration of the sugar solution. Urine volume for each period was recorded, and a 10 ml aliquot was saved, preserved by addition of 10 µl of NaN_3 (0.1 g/L) solution, and frozen at –70 C. After completion of our study, all samples were shipped on dry ice from the Hill's Pet Care Center to the gastrointestinal laboratory and after arrival were stored frozen at –80 C until analysis.

Analysis of the urine samples was performed as described.[2] Briefly, immediately prior to analysis samples were thawed and mixed, and a 1-ml aliquot was filtered through a 0.1-µm-diameter pore syringe filter. Each sample was diluted 1 to 100 with deionized water with 0.1 g/L NaN_3, and 25 µl was injected into the high pressure liquid chromatography system[f-h] with pulsed amperometric detection.[i] The detector signal was digitized, and a chromatogram was generated by use of a computer software package.[j] The UR for each sugar and time point (ie, each 2-hour interval) was calculated by the following equation:

$$UR = (\text{sample concentration} \times 100 \times \text{urine volume})/(\text{sugar mass administered}).$$

Percent urinary recovery (%UR) for each sugar at each time point was calculated by the following equation:

$$\%UR = (UR \times 100)/(\text{cumulative UR after 12 hours}).$$

Results

At the conclusion of our study, 2 of 7 dogs developed mild and transient diarrhea. Diarrhea lasted for 24 to 48 hours and resolved without intervention.

Mean (± SD) UR of sugars 12 hours after orogastric intubation was 6.1 ± 6.2% for lactulose, 36.0 ± 9.3% for rhamnose, 31.5 ± 7.9% for xylose, 83.2 ± 19.1% for methylglucose, and 1.1 ± 1.9% for sucrose. A high degree of variability (coefficients of variation: lactulose recovery, 101.6%; rhamnose recovery, 25.8%; xylose recovery, 25.1%; methylglucose recovery, 23.0%; and sucrose recovery, 172.7%) was found in the total 12-hour UR among sugars.

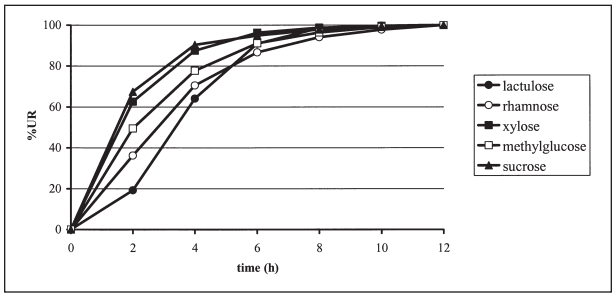

Figure 1—Mean percentage of urinary recovery (%UR) of lactulose, rhamnose, xylose, methylglucose, and sucrose at various time points after orogastric administration in 7 healthy dogs.

Mean %UR (**Fig 1**; **Table 1**) was ≥ 70.3% (mean, 79.4%) for all 5 sugars at 4 hours after administration, ≥ 85.1% (mean, 90.3%) at 6 hours, ≥ 93.4% (mean, 96.2%) at 8 hours, and ≥ 98.4% (mean, 99.0%) at 10 hours. The SD of the mean %UR for all 5 sugars was ≤ 43.3% (mean, 28.1%) 2 hours after sugar administration, ≤ 8.1% (mean, 6.8%) after 4 hours, ≤ 8.0% (mean, 6.4%) after 6 hours, ≤ 4.2% (mean, 2.7%) after 8 hours, and ≤ 1.7% (mean, 1.3%) after 10 hours.

Discussion

The purpose of our study was to describe the kinetics of UR of lactulose, rhamnose, xylose, methylglucose, and sucrose after orogastric intubation in healthy dogs. All 7 dogs used in our study belonged to a well-maintained research colony housed at and owned by the Hill's Pet Care Center. Dogs were monitored daily, and clinical signs suggestive of gastrointestinal tract disease were not observed. Results of routine laboratory tests also did not reveal any noteworthy abnormalities. Although subclinical gastrointestinal tract disease was unlikely, we were unable to conclusively exclude the presence of such in the dogs of our study.

Total UR among the 5 sugars 12 hours after administration differed largely. This is not surprising, because the 5 sugars traverse the gastrointestinal mucosa by differing mechanisms. Sucrose permeates the gastric mucosa, and lactulose and rhamnose pass through the gastrointestinal mucosa by way of aqueous pores. Some authors hypothesize that lactulose passes through large aqueous pores that are located paracellularly and that are found infrequently.[1,19,20] In contrast, rhamnose is hypothesized to permeate the gastrointestinal mucosa through small aqueous pores that are located transcellularly and are found at a high frequency.[1,19,20] Therefore, the permeability for rhamnose in dogs with a normally functioning gastrointestinal tract is high, whereas the permeability for lactulose is low. Sucrose is hydrolyzed to glucose and fructose as soon as it reaches the small intestine.[4] Thus, sucrose can only permeate through infrequently found large pores in the stomach or the proximal portion of the duodenum.[4] Xylose and methylglucose are absorbed through carrier-mediated transport processes.[10] Different carriers are most

Table 1—Mean (± SD) percent urinary recovery (%UR) of lactulose, rhamnose, xylose, methylglucose, and sucrose at various time points after orogastric administration in 7 healthy dogs

	%UR				
Time (h)	**Lactulose**	**Rhamnose**	**Xylose**	**Methylglucose**	**Sucrose**
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2	37.5 ± 24.5	38.7 ± 21.9	59.4 ± 27.5	47.6 ± 23.3	67.5 ± 43.3
4	71.3 ± 8.1	70.3 ± 6.8	88.6 ± 5.2	76.5 ± 5.8	90.4 ± 7.9
6	87.6 ± 8.0	85.1 ± 7.0	95.6 ± 5.1	88.3 ± 5.3	94.9 ± 6.8
8	95.8 ± 4.2	93.4 ± 2.6	99.0 ± 0.9	94.7 ± 2.1	97.9 ± 3.5
10	98.8 ±1.7	98.4 ± 1.7	99.8 ± 0.3	98.6 ± 1.4	99.5 ± 1.3
12	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

likely involved in the absorption of both of these sugar markers, which would also explain the difference in UR between xylose and methylglucose observed in our study.

At the conclusion of our study, 2 of 7 dogs had developed diarrhea. It has previously been reported that hyperosmolar solutions can alter gastrointestinal permeability.[21,22] In 1 study,[21] sugar solutions with an osmolality of 2,000 mOsm/kg were used. Also, other authors stated that osmolalities in excess of 1,500 mOsm/kg are needed to alter permeability.[1] Although a potential increase in gastrointestinal permeability may have affected the total UR of the 5 sugars examined in our study, the kinetics of UR should not have been affected. After completion of our study, the protocol for all further studies was changed, and sugar solutions were prepared to be iso-osmolal.

Results of a recent study[k] to determine permeability and absorption values by use of a sugar solution with an osmolality of 295 mOsm/kg in 23 healthy dogs revealed 6-hour UR values that were extremely similar to the values found in our study. Comparison of findings between our study and the previous study[1] indicates that the hyperosmolality of the sugar solution used in our study had no influence on UR or kinetics of UR of the 5 sugars.

Lactulose is used as an osmotic laxative. However, the therapeutic dose of 330 mg/kg (total calculated dose for the dogs used in our study was approx 4.7 to 6.2 g) recommended in dogs is higher than the 2.5 g/dog administered in our study. Furthermore, even though the osmolality of the solution administered by orogastric intubation was 745 mOsm/kg, the osmolality in the small intestine is unknown because sucrose is hydrolyzed and the resulting glucose and fructose are absorbed quickly after the solution reaches the duodenum. Despite this, we concluded that although an influence on permeability and absorption of the 5 sugars was unlikely, the hyperosmolality of the sugar solution was most likely responsible for the mild and transient diarrhea, and as mentioned, isotonic sugar solutions were used for all further experiments.

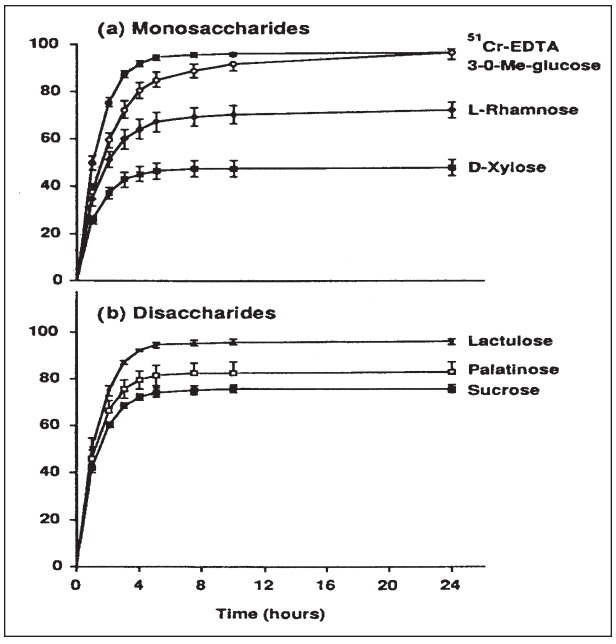

Figure 2—Mean urinary recovery (UR) after IV administration of selected monosaccharides and disaccharides and ^{51}Cr-EDTA. Figure reprinted with permission from Hall EJ, Batt RM. Urinary excretion by dogs of intravenously administered simple sugars. *Res Vet Sci* 1996;60:280–282.

A high degree of variability between dogs was found in the total 12-hour UR for all 5 sugars in our study. One possible reason for this variability could be the small number of dogs (n = 7) that was used in our study. Also, although unlikely, some of the dogs may have had subclinical gastrointestinal tract disease leading to an increased permeability of lactulose and sucrose, a decreased permeability of rhamnose, and a decreased absorptive capacity for xylose and methylglucose. Although subclinical gastrointestinal tract disease may cause slight alterations in UR of sugars during a 12-hour period, it should not alter gastrointestinal motility substantially and should, therefore, not affect the kinetics of urinary excretion.

Mean %UR (the % of the sugar recovered up to a certain time point compared with the total recovery of the sugar recovered after 12 hours; Table 1) of all sugars increased with time, and the SD of the mean %UR decreased with time. Although there appears to be no substantial decrease of the SD after 4 hours, the mean %UR after 4 hours is only 79.4%, whereas it was 90.3% after 6 hours. Thus, a substantial amount of the 5 sugars is recovered between 4 and 6 hours after orogastric administration. These results suggest that urine collection for only 4 hours is not sufficient for gastrointestinal permeability and mucosal function testing in dogs. This is especially true because the dogs enrolled in our study did not have overt gastrointestinal tract disease, and dogs with gastrointestinal tract disease may have altered gastrointestinal motility leading to either faster or slower recovery of sugars after orogastric administration.

In a study[18] on the UR of sugars and ^{51}Cr-EDTA after IV administration, UR of the same 5 sugars examined in our study was almost complete 7.5 hours after IV administration (lactulose, 99.3%; rhamnose, 96.3%; xylose, 98.5%; methylglucose, 91.8%; and sucrose, 99.2%; **Fig 2**).[18] In that study,[18] a 6-hour measurement was not made, but UR was > 90% for all 5 sugars at 5 hours after IV administration (lactulose, 98.3%; rhamnose, 93.2%; xylose, 97.3%; methylglucose, 87.9%; and sucrose, 97.9%).[18] These values are higher than the 6-hour %UR values in our study. However, passage through the gastrointestinal tract, permeation through or absorption by the gastrointestinal mucosa, and distribution into the vascular space require additional time. Time required for these steps results in a delay of urinary excretion of sugar markers when given by orogastric intubation, compared with IV administration. On the basis of findings in our study, we conclude that urine collection for 6 hours is sufficient to accurately assess gastrointestinal permeability and mucosal function following lactulose, rhamnose, xylose, methylglucose, and sucrose orogastric administration in healthy dogs.

[a]L-7877, Sigma Chemical Co, St Louis, Mo.
[b]R-3875, Sigma Chemical Co, St Louis, Mo.
[c]X-1500, Sigma Chemical Co, St Louis, Mo.
[d]M-4879, Sigma Chemical Co, St Louis, Mo.
[e]S-7903, Sigma Chemical Co, St Louis, Mo.
[f]717+ Autosampler, 625 pump, Waters Corp, Milford, Mass.
[g]Carbopac™ PA10 analytic and guard columns, DIONEX Corp, Sunnyvale, Calif.
[h]Duros CC-30-S-PK, ELDEX Laboratories, Napa, Calif.
[i]Electrochemical detector 464, Waters Corp, Milford, Mass.
[j]Millenium 32 chromatography manager, Waters Corp, Milford, Mass.
[k]Steiner JM, Cardwell JR, Williams DA. Determination of a control range for a 5-sugar gastrointestinal permeability and mucosal function test in clinically healthy dogs (abstr). *J Vet Intern Med* 2001;15:311.

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