Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal of Virological Methods, 18 (1987) 153–157
Elsevier

JVM 00655

Assessment of intestinal damage in rotavirus infected neonatal mice by a D-xylose absorption test

M.K. Ijaz[1], M.I. Sabara[1,2], P.J. Frenchick[1] and L.A. Babiuk[1,2]

[1]*Veterinary Infectious Disease Organization (VIDO) and* [2]*Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

(Accepted 1 July 1987)

Summary

A D-xylose absorption test has been standardized for use in newborn mice. It was used to measure small intestine dysfunction in neonate mice following infection with various isolates of rotaviruses. A xylose dose of 1 mg/g body weight was required to produce a maximum level of 100 μg/100 μl of plasma 2 h after administration of D-xylose. The mice inoculated with rotavirus absorbed significantly less D-xylose compared with uninoculated control mice. A micromethod is described which proved to be suitable to quantitate D-xylose for determination of absorptive function of the small intestine in small animals such as newborn mice.

Animal model; Rotavirus; D-Xylose test

Rotavirus has been identified as the single most important cause of enteritis in neonates of most species of domestic and laboratory animals and humans. Most of the isolates have been shown to cross-infect and produce diarrhea in different species of mammals. Since animals infected with virus show atrophy of the villi in the small intestine with a decrease in absorption, assessment of absorptive dysfunction should correlate well with clinical disease (Flewett and Babiuk, 1984; Babiuk et al., 1985).

Published with the permission of the Director of VIDO as Journal Series No 57.

Correspondence to: Dr. M.K. Ijaz, Veterinary Infections Disease Organization, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 0W0.

In order to monitor the effectiveness of different anti-rotavirus agents, it is of paramount significance to assess the degree of intestinal damage caused by the virus. The D-xylose absorption test has been used as an index of intestinal absorptive function in humans (Mavromichalis et al., 1977), dogs (Hill et al., 1970), horses (Breukink et al., 1974; Roberts, 1974; Bolton et al., 1976; Meuten et al., 1978; Jacobs et al., 1982), cattle (Pearson and Baldwin, 1981), cats (Emms et al., 1983), fowl (Goodwin et al., 1984; Yason and Schat, 1986), rats and mice (Henegham, 1963). However, its use in virus infections has been limited to children, calves and birds suffering from rotavirus associated diarrhea (Mavromichalis et al., 1977; Woode et al., 1978; Yason and Schat, 1986). Since a mouse model is being used extensively to study the pathogenesis and prevention of rotavirus-induced diarrhea, it was advantageous to evaluate this test in neonate mice. We have modified a D-xylose absorption test (Eberts et al., 1979) to study the extent of malabsorption through intestine in neonate mice infected with various isolates of rotaviruses.

Three groups of mice (5 per group) 7 days old were selected for D-xylose (Sigma, St. Louis, MO, U.S.A.) absorption studies. Group 1 acted as control whereas group 2 and 3 received bovine rotavirus (BRV) and mouse rotavirus (MRV), respectively. The procedure for the preparation of BRV and MRV stocks used in the challenge have been described previously (Ijaz et al., unpublished data). Control groups were given only xylose in double distilled water. The preparations were administered by intubation of the stomach with a soft flexible plastic feeding tube. Twenty-four hours after infection a 5% w/v solution of D-xylose (100 μl/mouse) in deionized water was administered by the feeding tube. Two hours after adminis-

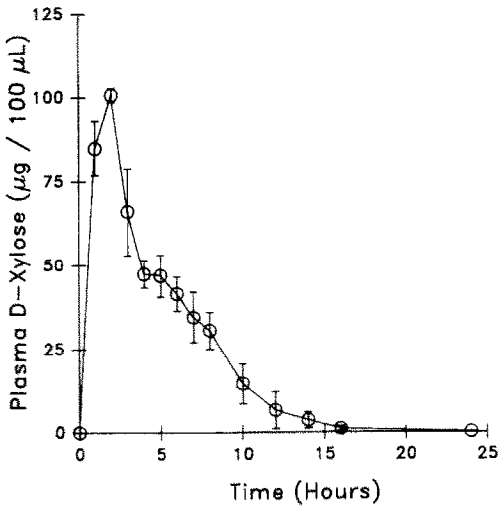

Fig. 1. Kinetics of D-xylose absorption in rotavirus-free neonatal mice. A 5% w/v solution of D-xylose (100 μl/mouse) was administered to neonatal mice. Three mice at each time interval were sacrificed by decapitation and their blood was collected using heparinized hematocrit tubes. Plasma was collected by centrifugation of the blood-filled hematocrit tubes and assayed for D-xylose concentration as described in the text. The blood from uninoculated (xylose) neonates served as negative control.

tration of D-xylose, the animals were killed and blood samples from each were collected using heparinized hematocrit tubes which were centrifuged to yield plasma.

For determination of plasma D-xylose concentration a modified micromethod, as reported by Eberts et al. (1979) was used. One ml of phloroglucinol (1,3,5-trihydroxybenzene, Sigma Chemical Co., St. Louis, MO) reagent (0.5 g of phloroglucinol, 100 ml of glacial acetic acid and 100 ml of conc.HCl) was added to 10 µl of plasma. This solution was heated to 100°C in a water bath for 4 min to allow optimum color development. After equilibration to room temperature, sample absorption was determined with the aid of a spectrophotometer (Ultraspec, LKB Biochem Ltd., Cambridge, U.K.) set at a wavelength of 554 nm.

The kinetics of D-xylose absorption in normal newborn mice is presented in Fig. 1. As indicated in the figure, maximum absorption of D-xylose takes place 2 h after administration of D-xylose. Therefore, the sampling time chosen to collect blood samples in subsequent experiments was 2 h post-administration. Fig. 2 shows results of intestinal D-xylose absorption experiments with normal and rotavirus-inoculated neonates. In all cases, rotavirus-inoculated mice had lower mean plasma D-xylose concentrations. Within the two virus inoculated groups the one inoculated with the more virulent virus, revealed the least absorption of D-xylose. None of the control mice receiving D-xylose alone developed alimentary discomfort or diarrhea.

It has been shown experimentally, in gnotobiotic calves, pigs and mice infected with rotavirus that the histopathology of atrophy of the villi in the small intestine is similar to that seen following natural infection (Babiuk et al. 1985). Therefore, this test has been regarded as an indirect measure of the loss of these cells (Woode

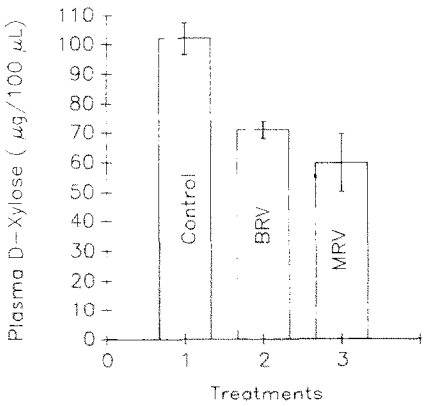

Fig. 2. Comparison of plasma D-xylose concentration between control and rotavirus-inoculated mice. Animals were inoculated with either bovine or mouse rotavirus whereas controls received dH_2O (five mice were included for each treatment). Twenty-four hours after virus-inoculation they were given D-xylose (100 µl of 5% w/v solution of D-xylose). Two hours after administration of xylose they were sacrificed by decapitation and their blood was collected using heparinized hematocrit-tubes. Plasma was collected by centrifugation of the blood-filled hematocrit tubes and assayed for D-xylose concentration as described in the text.

et al., 1978). Based on this fact the results of this study indicate that more damage is done by MRV than by BRV in the mouse model used. Earlier experiments carried out also revealed MRV to be more virulent than BRV in this model (M.K. Ijaz, et al., in preparation).

At necropsy, neonates infected with rotavirus had frothy fluid in the large intestine, which may be a direct consequence of damage to the epithelial cells of the small intestine. Damaged cells may be replaced by immature cells unable to provide the enzymes needed for the final digestion and absorption of carbohydrates and sugars, as suggested by the D-xylose test. Unabsorbed nutrients may undergo bacterial fermentation in the intestine, producing osmotically active metabolites. These products may then cause the fluid-filled distension of the large intestine by osmosis (Abrams et al., 1963; Abrams and Bishop, 1967).

D-xylose is absorbed mainly by passive diffusion from the small intestine (Roe and Rice, 1948). It may also be absorbed to a lesser extent by the active transport system that is responsible for absorbing glucose and galactose. Since active transport has a preference for glucose, xylose is absorbed at a much slower rate (Meuten et al., 1978). Thus, the presence of glucose in the lumen of the small intestine will inhibit the absorption of xylose by active transport, but not affect passive absorption. Therefore, before conducting D-xylose test, animals must be fasted at least 12 h prior to administration of D-xylose.

Xylose, being a pentose sugar, is partly transported actively like glucose, and therefore appears to be a reliable indicator of malabsorption in the proximal small intestine (Eberts et al., 1979). The results obtained by this micromethod are comparable to the one obtained by high-performance liquid-chromatographic separation technique (Eberts et al., 1979). The most obvious advantage of this method is the small plasma sample size (50 μl) and the short incubation time (4 min) required. This study modified the procedure further to reduce the required plasma sample to 10 μl, allowing quantitation of intestinal D-xylose absorption in extremely young and small animals such as newborn mice.

Acknowledgements

We would like to acknowledge the excellent technical assistance of Donna Dent and Barry Carroll. We also thank Irene Kosokowsky for typing the manuscript. Financial support was provided by the Medical Research Council and the Natural Sciences and Engineering Council of Canada.

References

Abrams, G.D., Bauer, H. and Spring, H. (1963) Influence of the normal flora on mucosal morphology and cellular renewal in the ileum: a comparison of germ-free and conventional mice. Lab. Invest. 12, 355–364.

Abrams, G.D. and Bishop, J.E. (1967) Effect of the normal microbial flora on gastrointestinal motility. Proc. Soc. Exp. Biol. Med. 126, 301–304.

Babiuk, L.A., Sabara, M. and Hudson, G.R. (1985) Rotavirus and coronavirus infections in animals. Prog. Vet. Microbiol. Immun. 1, 80–120.

Bolton, J.R., Merritt, A.M., Cimprich, R.E., Rambert, C.F. and Streett, W. (1976) Normal and abnormal xylose absorption in the horse. Cornell Vet. 66, 183–197.

Breukink, H.J. (1974) Oral mono- and disaccharide tolerance tests in ponies. Am. J. Vet. Res. 35, 1523–1527.

Eberts, T.J., Samle, R.H.B., Glick, M.R. and Ellis, G.H. (1979) A simplified colorimetric micromethod for xylose in serum or urine, with phloroglucinol. Clin. Chem. 25, 1440–1443.

Emms, S.G., Hawkins, C.D and Clark, W.T. (1983) The rate of D-xylose absorption in normal cats. Aust. Vet. J. 60, 30–31.

Flewett, T.H. and Babiuk, L.A. (1984) Prospects for rotavirus vaccine in humans and animals. In: Control of Virus Diseases. (Kurstak, E. and Marusyk, R.G., eds.), pp. 57–65, Marcel Dekker, Inc., N.Y.

Goodwin, M.A., Latimer, K.S., Nersessian, B. and Fletcher, O.J. (1984) Quantitation of intestinal D-xylose in normal and reovirus-inoculated turkeys. Avian Dis. 28, 959–967.

Heneghan, J.B. (1963) Influence of microbial flora on xylose absorption in rats and mice. Am. J. Physiol. 205, 417–420.

Hill, F.W.G., Kidder, D.E. and Frew, J. (1970) A xylose absorption test for the dog. Vet. Rec. 87, 250–255.

Jacobs, K.A., Norman, P., Hodgson, D.R.G. and Cymbaluk, N. (1982) Effect of diet on the oral D-xylose absorption test in the horse. Am. J. Vet. Res. 43, 1856–1858.

Mavromichalis, J., Evans, N., McNeish, A.S., Bryden, A.S., Davies, H.A. and Flewett, T.H. (1977) Intestinal damage in rotavirus and adenovirus gastroenteritis assessed by D-xylose malabsorption. Arch. Dis. Child. 52, 589–591.

Meuten, D.J., Butler, D.G., Thomson, G.W. and Lumsden, J.H. (1978) Chronic enteritis associated with malabsorption and protein losing enteropathy in the horse. J. Am. Vet. Med. Assoc. 172, 326–333.

Pearson, E.G. and Baldwin, B.H. (1981) D-xylose absorption in the adult bovine. Cornell Vet. 71, 288–296.

Roberts, M.C. (1974) The D(+) xylose absorption test in the horse. Equine Vet. J. 6, 28–30.

Roe, J.H. and Rice, E.W. (1948) A photometric method for the determination of fine pentoses in animal tissue. J. Biol. Chem. 173, 507–512.

Woode, G.N., Smith, C. and Dennis, M.J. (1978) Intestinal damage in rotavirus infected calves assessed by D-xylose malabsorption. Vet. Rec. 102, 340–341.

Yason, C.V. and Schat, K.A. (1986) Pathogenesis of rotavirus infection in turkey poults. Avian Pathol. 15, 421–435.