Bile Acid Malabsorption in Cystic Fibrosis; Membrane Vesicles, a Tool for Revealing the Role of the Ileal Brush Border Membrane

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ABSTRACT. de Rooij, F. W. M., van den Berg, J. W. O., Sinaasappel, M.,[1] Bosman-Jacobs, E. P. and Touw-Blommesteijn, A. C. (Department of Internal Medicine II and [1]Department of Pediatrics, subdivision Gastroenterology, Erasmus University Rotterdam, 3000 DR Rotterdam, The Netherlands). Bile acid malabsorption in cystic fibrosis; membrane vesicles, a tool for revealing the role of the ileal brush border membrane. Acta Paediatr Scand Suppl 317.

Increased fecal bile acid loss in cystic fibrosis (CF) may result from ileal dysfunction. A method to quantitate in vitro Na^+-dependent taurocholate uptake into brush border membrane vesicles prepared from frozen ileum and ileal biopsy specimen is described. This transport across the ileal brush border membrane can be measured selectively, in contrast to in vivo measurements which represent a complex overall process. Preliminary results obtained with ileal specimen of 2 CF patients, suggest that in vitro bile acid uptake is low but not abnormal. ***Key words: Bile acids, brush border membrane, cystic fibrosis, ileal transport.***

Increased fecal bile acid loss is found in a variety of gastrointestinal diseases and bile acid malabsorption may result from ileal dysfunction. In cystic fibrosis (CF) increased fecal bile acid loss is also one of the phenomena (1); furthermore a decreased bile acid pool is found in CF (2). Fondacaro et al. (3) suggested that reduced bile acid absorption in the terminal ileum of CF patients may cause bile acid loss in CF. Although bile acids are absorbed by passive diffusion, most important is the Na^+-dependent active absorption of conjugated bile acids (against a concentration gradient) in the terminal ileum. The more viscous mucus layer, as found in CF, may be a factor in diminished bile acid absorption as well. We have been able to characterize the ileal Na^+-dependent taurocholate transport across the brush border membrane in vitro by preparing brush border membrane vesicles (BBMV) from microquantities (25–200 mg) of ileum, allowing the use of biopsy specimen (4). BBMV were prepared according to an adaptation of the method of Kessler et al. (5). Vesicles, prepared in this way from enterocyte brush border membranes, do have the »right side out» configuration. The outer side of those BBMV normally is exposed to the luminal content. The absorptive capacity of the ileal enterocytes for specific components like bile acids can be studied in vitro, separated from the complex in vivo situation. In this study uptake of ^3H-taurocholate into the BBMV in the presence of a 100 mM NaCl gradient is followed during 1 min. Samples were taken at 20, 40 and 60 sec after starting the incubation and uptake was related to the amount of membrane protein.

MATERIALS AND METHODS

Brush border membrane vesicle isolation

Brush border membrane vesicles (BBMV) were prepared (at 0–4°C) according to an adaptation of the procedure of Kessler et al. (5), based on a precipitation with divalent cations. Frozen (−70°C) ileal specimen (25–200 mg) obtained after resection, obduction or as endoscopical biopsy were thawed in isotonic buffer (300 mM mannitol, 12 mM Tris-HCl, pH 7.1) at a final concentration up to 250 mg/ml. Disruption of the material was carried out in an "Eppendorf" tube in a minimal buffer volume of 150

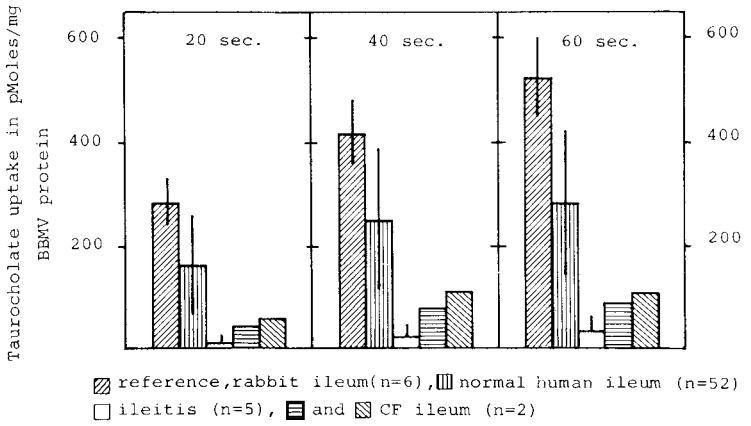

Fig. 1. Taurocholate uptake into brush border membrane vesicles (BBMV). BBMV were prepared from microquantities of rabbit ileal segments of from colonoscopical biopsy specimen, histologically diagnosed as normal ileum or ileitis, and from two surgical specimen from CF patients. The standard deviation is indicated.

μl for two 45 sec periods using a Vibro-mixer (Model E1 from Chem. Ap. A. G. Mannedorf Z.H., Switzerland) fitted with a glass rod. If present, the suspension was cleared from small pieces of muscle and connective tissue using a microliter pipette. Then, the suspension was diluted with 5 volumes of icecold water and $MgCl_2$ was added to a final concentration of 10 mM. After 40 min the suspension was centrifuged at 3000 × g for 15 min. The supernatant was decanted into another tube and stored at 0°C. The pellet was resuspended in buffer (50 mM mannitol, 2 mM Tris-HCl, 10 mM $MgCl_2$, pH 7.1) and after 40 min this suspension was centrifuged as before. The pellet was discarded. The two supernatant fractions were combined and centrifuged for 30 min at 27000 × g to spin down the BBMV. The final BBMV pellet was resuspended in 34 μl buffer (100 mM mannitol, 1 mM HEPES-Tris, 10 μM $MgSO_4$, pH 7.5) using a "Hamilton" syringe. Protein content was determined in duplicate on 6 μl samples (6).

Transport measurements

The BBMV suspension (17 μl) was preincubated for 5 min at 25°C and the incubation was started by addition of 204 μl of transport buffer (100 mM mannitol, 108 mM NaCl, 1 mM HEPES-Tris, 10 μM $MgSO_4$, pH 7.5) containing 4 μM ^{3}H-taurocholate (6.6 Ci/mmol New England Nuclear Corp.). Samples (50 μl) of the incubation mixture were taken at selected times (20, 40 and 60 sec) and diluted in 4 ml icecold stop buffer (100 mM mannitol, 100 mM NaCl, 1 mM HEPES-Tris,10 μM $MgSO_4$, pH 7.5 saturated with lithocholate (≤50 μM)), filtered over a membrane filter (Sartorius SM 11306, 0.45 μM pore size) on a filtration unit (Amicon Manifold, pressure difference 650–700 mmHg) and washed 4 times with 4 ml of the same stop buffer. Each membrane filter was dissolved in 10 ml scintillation liquid. Radioactivity was measured in a liquid scintillation counter.

RESULTS

The microprocedure was validated on both fresh frozen rabbit ileum and human obduction ileum (4). Uptake determinations were performed with colonoscopical ileal biopsy specimen histologically diagnosed as normal (n=52) and ileitis (n=5), as well as with ileal specimen of two CF patients. The CF specimen were taken from the resection area during the closure of an ileostomy. We used frozen rabbit ileum as a reference for our in vitro assay (see Fig. 1).

DISCUSSION

Increased fecal bile acid loss in CF may be due to a reduced or absent active ileal transport (3). The brush border membrane plays a major role in the Na^+-dependent active bile acid

transport of the ileum. Therefore we studied the integrity of this process in two CF patients. The assay described here has already shown its diagnostic value for patients with increased fecal bile loss of unknown origin. In five patients with histologically proven ileitis and with increased fecal bile acid loss, a decreased in vitro taurocholate uptake was found. The uptake measured in this assay represents only the active bile acid uptake capacity of brush border membranes. Altered viscosity of mucus, as found in CF, does not affect our results because no mucus layer is present on the BBMV. Moreover, due to the low bile acid concentration used in the assay, the uptake is not disturbed by a contribution of the passive diffusion of bile acids. Preliminary results obtained with ileal specimen of 2 CF patients suggest that in vitro bile acid uptake is low but not abnormal.

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