Treatment of Bile Acid Malabsorption Using Ileal Stem Cell Transplantation

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BACKGROUND: We hypothesized that ileal stem cell clusters transplanted into a segment of jejunum can be used to treat bile acid malabsorption.

STUDY DESIGN: In adult Lewis rats, a 15-cm segment of jejunum was isolated with its blood circulation left intact and partially stripped of enterocytes using luminal high-velocity perfusions with 3 mmol/L ethylenediamine tetra-acetic acid solutions. Continuity was restored by anastomosing the proximal and distal gut. Ileal stem cell clusters were harvested from neonatal Lewis rats and transplanted into the stripped segments to generate a "neoileum." After 4 weeks, recipients underwent resection of the native ileum, and the isolated neoileum was anastomosed in its place. After an additional 4 weeks, a 48-hour stool collection was performed. The engrafted segment was harvested for taurocholate uptake studies, ileal bile acid transporter (IBAT) protein by immunohistomorphometry, and IBAT mRNA quantitation by reverse transcription polymerase chain reaction. Data were analyzed by ANOVA/*t*-test. Rats undergoing ileectomy, jejunectomy, or sham operations served as controls.

RESULTS: Total bile acid loss in the stool was markedly lower in rats with a neoileum compared with rats with an ileectomy ($p < 0.001$). Total taurocholate uptake was notably increased in the neoileum compared with the jejunum ($p < 0.001$). IBAT protein signal intensity was considerably higher in the neoileum compared with jejunum ($p < 0.001$). IBAT mRNA amounts in the neoileal group were comparable with those in normal rat ileum and were considerably higher ($p = 0.003$) than in the jejunum.

CONCLUSIONS: Ileal stem cell clusters were used to establish a new zone of bile acid uptake and IBAT expression in a jejunal segment. This neoileum eliminated loss of bile acids in the stool after ileectomy. This is the first time that transplantation of intestinal stem cell clusters has been shown to correct a clinical malabsorption syndrome. (J Am Coll Surg 2005;201:710–720. © 2005 by the American College of Surgeons)

Bile acid malabsorption is a common form of intestinal malabsorption caused by anatomic, inflammatory, physiologic, or molecular changes in the ileum of the small intestine that functionally decreases the ability of the enterohepatic circulation to recover bile acids. This can occur after surgical resection of the ileum, inflammatory diseases such as Crohn's disease, radiation enteritis, or terminal ileitis, which alter the function of the enterocytes, cause changes in motility that decrease transit time, or bring about defects in membrane transporters responsible for bile acid absorption. This can occur after surgical resection of the ileum and with inflammatory diseases such as Crohn's disease, radiation enteritis, or terminal ileitis. These processes can alter the function of the enterocytes, cause changes in motility that decrease transit time, or bring about defects in membrane transporters resonsible for bile acid absorption. Resection of as little as 30 cm of the distal half of the ileum may precipitate bile acid malabsorption. Bile acid absorption is also decreased in patients with radiation enteritis, cystic fibrosis, or Crohn's colitis whose ileum is histologically normal.[1] Increased exposure of the colonic entero-

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Abbreviations and Acronyms

DMEM-S = Dulbecco's modification of Eagle's medium – sterile
EDTA = ethylenediamine tetra-acetic acid
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
HBSS = Hank's buffered saline solution
IBAT = ileal bile acid transporter
PCR = polymerase chain reaction

cytes to higher concentrations of bile acids can result in colonic enterocyte dysfunction, causing diarrhea. In addition, elevated levels of bile acids may have a mutagenic effect on colonic enterocytes and have been associated with increased risk of colon cancer.[2,3] Other serious conditions such as gallstones, nephrolithiasis, growth retardation, and weight loss can also arise.[4-6] Successful transplantation of ileal stem cell clusters into the jejunum could be used to treat bile acid malabsorption by generating a segment with neoileal mucosa.

Stem cells of the small intestine are defined by their ability to produce all of the epithelial lineages of the small intestine (ie, enterocytes, Paneth cells, goblet cells, and enteroendocrine cells). Pluripotency of the cells and proliferative capacity are greatest at the crypt base, decreasing as cells move upwards. Stem cells divide to produce daughter cells, known as transit amplifying cells. These proliferate and differentiate, rapidly appearing on the villus as terminally differentiated cells.[7] Isolation and manipulation of individual intestinal stem cells have failed to give rise to a functional neomucosa.[8] Mesenchymal cells appear to be important in regulating and maintaining endodermal differentiation, suggesting a role in the regulation of epithelial stem cell function. Tait and colleagues[8] described a method in which aggregates of neonatal rat epithelial and mesenchymal intestinal cells (which contain intestinal stem cells) can be isolated and used to generate a neomucosa. By definition, the formation of crypts and villi in this neomucosa can only be derived from stem cell progenitors.

Our group first reported successful transplantation of ileal stem cell clusters into denuded jejunal segments in 2000.[9] This was accomplished by isolating stem cell clusters from terminal ileum of neonatal rats and transplanting them into demucosalized segments of midjejunum in adult syngeneic recipients. The "neoileum" was identified using antibodies to the ileal bile acid transporter (IBAT) protein, which is predominately located on mature ileocytes and is responsible for sodium-dependent uptake of bile acids. Active taurocholate uptake was demonstrated in segments with neoileal mucosa, but rates were lower than those seen in the native ileum. IBAT-mRNA was transcribed in the neoileum at levels 18- to 100-fold higher than those normally detected in the jejunum.

This study examined whether similar neoileal segments can reverse bile acid malabsorption after ileectomy in vivo. We hypothesized that a neoileum placed in continuity with the small intestine after an ileectomy would reverse bile acid loss in the stool and restore normal stool consistency.

METHODS

Animals

Five- to 7-day-old Lewis rats were used as donors for intestinal stem cell cluster isolation. Adult male Lewis rats weighing 220 to 250 g were used as syngeneic recipients (Harlan). All animals were housed in accordance with the National Institutes of Health guidelines for the care of laboratory animals, maintained under a 12-hour light/dark cycle (light from 6 AM to 6 PM), and received regular rodent chow and water ad libitum.

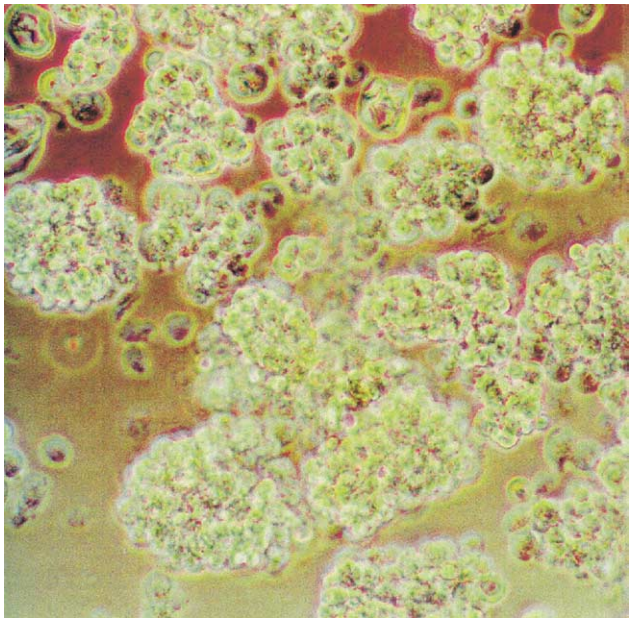

Figure 1. Ileal stem cell clusters containing 20 to 40 cells from the base of crypts of rats.

Isolation of stem cell clusters

Intestinal stem cell clusters were isolated as described by Evans and associates[10] (Fig. 1). In brief, 5- to 7-day-old rats were sacrificed by cervical dislocation and the terminal one-fifth of the small bowel (terminal ileum) was harvested. The intestine was opened longitudinally and cut into 2-mm long segments. The intestinal pieces were washed in calcium-free and magnesium-free Hank's buffered saline solution (HBSS, Mediatech Inc). The washed segments were minced into pieces smaller than 1 mm^3, then transferred into 20 mL of HBSS, with 0.1 mg/mL dispase type 1 (Roche) and 300 U/mL collagenase type XI (Sigma) and rocked continuously for 25 minutes at 22°C. The suspension was shaken vigorously for 1 minute and allowed to sediment for 1 minute. The supernatant was carefully transferred to a new tube and the pellet discarded. This was repeated three times, clearing the suspension of larger debris. The cleared suspension then was mixed with an equal amount of sterile Dulbecco's modification of Eagle's medium (DMEM-S). The following was added to the 0.45% glucose DMEM-S (Gibco): 2% D-Sorbitol (Sigma), 2.5% fetal bovine serum (Hyclone), 100 IU/mL penicillin + 100 μg/mL streptomycin (Gibco). The modified DMEM-S/supernatant solution was centrifuged for 2 minutes at 300 rpm at room temperature. Contents were allowed to sediment at normal gravity for 30 seconds and the supernatant discarded. The pellet was washed (50 mL of DMEM-S) and centrifuged six times, or until the supernatant was clear and the pellet well defined. The pellet then was resuspended in HBSS containing calcium and magnesium.

Surgical procedure

First laparotomy

Adult syngeneic male Lewis rats were used as recipients of the stem cell clusters. Rats were anesthetized with inhaled isoflurane. A 15-cm segment of midjejunum was isolated in each rat on its mesenteric pedicle, maintaining perfusion to the segment. The remaining proximal and distal bowel was anastomosed in an end-to-end fashion to restore bowel continuity. All anastomoses were performed with 6-0 polypropylene sutures (Ethicon). The mucosal epithelium from the isolated jejunum was stripped by vigorous flushing with 0.9% saline containing 1 mmol/L dithiothreitol (5 minutes for mucolysis), 1 mmol/L dithiothreitol/27 mmol/L citrate (10 minutes) and 1 mmol/L dithiothreitol/3 mmol/L ethylenediamine tetraacetic acid (EDTA) (45 minutes) at a flow rate of 80 mL/min at 37°C.[11] This resulted in a partial dislodgment of the mucosal cells from the basement membrane with little damage to the underlying tissue. Before administration of the chelators, the mesenteric pedicle was clamped for the duration of the irrigation to minimize toxicity from chelators. Each segment was seeded with 100,000 to 130,000 ileal stem clusters in 1 mL of HBSS. The proximal end of the segment was tied off with a 6-0 polypropylene suture (Ethicon). The distal end of the segment was anastomosed with a 6-0 polypropylene suture in an end-to-side fashion to the distal ileum, just proximal to the ileocecal valve. The segments were placed in the abdomen for a 4-week period. The rats were maintained on microstabilized liquid rodent diet (Test Diet) for the first week and placed on normal rat chow (Nestle Purina) for the subsequent 3 weeks.

Second laparotomy

A midline laparotomy was performed. The intestines were exteriorized and adhesions were divided. The terminal 30 cm of ileum was removed. The proximal end of the neoileal segment was anastomosed to the proximal jejunum, restoring intestinal continuity. The rats were maintained on liquid diet for the first week and placed on normal rat chow for the subsequent 3 weeks. After this time, the rats underwent 48-hour stool collections. For this, rats were placed individually in metabolic cages. Stool and urine were collected continuously over a 48-hour period. Stool was stored at –80°C until the bile acid analysis was performed.

Experimental groups

Bile acid loss in the stool was determined in the following four groups: normal preoperative controls (n = 3); rats undergoing a jejunectomy (positive control; n = 3); rats undergoing an ileectomy (negative control; n = 3); and rats with neoileal segment (experimental group; n = 8) (Fig. 2). Rats with a jejunectomy had a 30-cm section of mid small bowel removed. The distal resection margin was 30 cm proximal to the ileocecal valve. Rats with an ileectomy had the terminal 30 cm of their small bowel resected. Neoileal intestinal tissue in the experimental group was compared with normal Lewis rat jejunum and

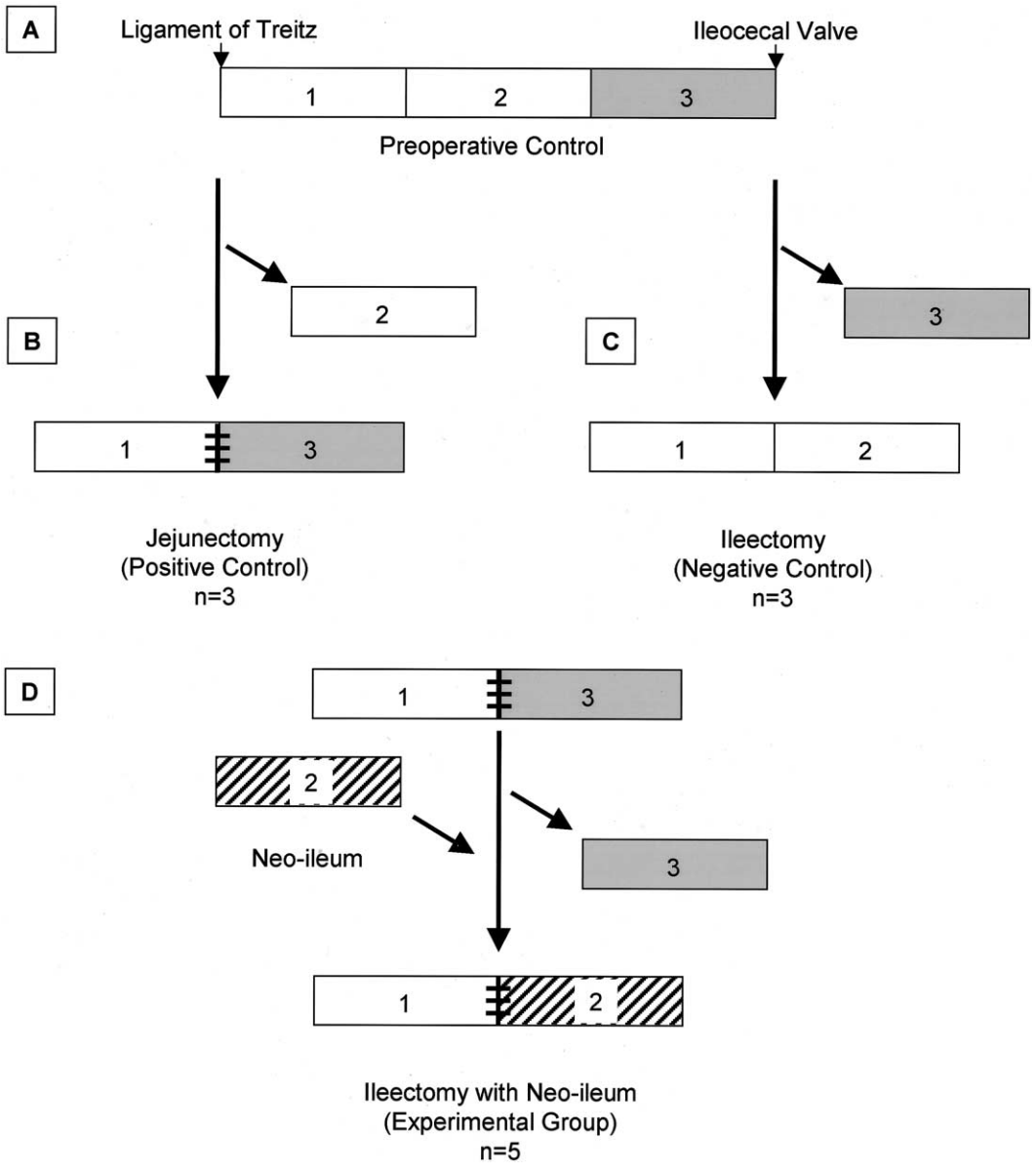

Figure 2. Bile acid excretion in the stool was determined in the following four groups: (A) intact small bowel (preoperative controls); (B) jejunectomy only (positive control); (C) ileectomy only (negative control); (D) neoileum (experimental group).

ileum for sodium-dependent bile acid uptake studies, tissue morphometry, and quantitation of IBAT mRNA and IBAT protein expression.

Bile acid uptake measurements

Sodium-dependent and sodium-independent bile acid absorption rates were measured in jejunal segments of rats that were seeded with ileal stem cells by a standardized everted sleeve method as previously described.[12,13] Briefly, tissue specimens were kept in 2°C oxygenated mammalian Ringer's solution. Specimens were then everted and secured onto 4-mm thick stainless steel rods using silk ties and stored in oxygenated ice-cold Ringer's solution. Uptake measurements were performed between 1 and 2 hours after excision. Mounted tissues were preincubated for 5 minutes in 37°C, oxygenated Ringer's solution and then incubated for 2 minutes in either sodium-containing or sodium-free Ringer's solution with

1 mmol/L taurocholate. Sodium-dependent transport was defined as the difference between uptake rates in sodium-containing and in sodium-free taurocholate-Ringer's solutions.

To measure sodium-independent uptake, sodium-free Ringer's solution was prepared by replacing sodium chloride with choline-Cl and sodium bicarbonate with potassium bicarbonate. Taurocholate in the sodium-free solutions was added as a potassium salt. To prepare potassium taurocholate, ion exchange of a 1M sodium taurocholate solution with potassium-loaded AmberLyte (Biorad) was performed. The potassium salt was subsequently recrystallized. Absence of sodium ions after the ion exchange was confirmed by flame-photometry. [^{3}H-(G)] Tracer amounts of radiolabeled taurocholic acid, (Perkin Elmer Life Sciences) was added to the uptake solutions. Polyethylene glycol, [1,2-^{14}C] (MW 4,000 Dalton), a marker substance not subject to carrier-mediated transport and with a very low transmembrane diffusion coefficient, also was added to these solutions to account for [^{3}H]-radiotracer in the adherent fluid.[13-15]

The length of the incubation period was chosen based on initial validation experiments using rat jejunal and ileal tissues as suggested by Karasov and Diamond.[15] These validation studies showed that, at 2 minutes, the uptake of radioactive taurocholate still showed linear increase over time, although complete equilibration of radioactive polyethylene glycol had occurred (data not shown).

After incubation, tissues were removed from the rods, placed in scintillation vials, weighed, and solubilized in 730 μL TS-2 tissue solubilizer (Research Products International). Vials were incubated at 58° C until the tissues were completely dissolved and 5 mL acidified Safety-Solve scintillation cocktail (Research Products International) was added. Beta emissions (in disintegrations per minute from tritium and ^{14}C were counted in a liquid scintillation counter (Tricarb 2200 CA, Packard Instrument Co). The average taurocholate uptake rate for the native ileum and jejunum was calculated for a 30- cm segment of bowel. The average taurocholate uptake rate for the neoileum was calculated for the 15 cm that was anastomosed in place of the resected ileum.

Immunohistochemistry

For immunohistochemistry studies, small intestinal tissues were mounted in optimal cutting temperature compound (Pella) and frozen on dry ice. Tissues were sectioned on a cryostat (Leica) and mounted on Superfrost/Plus glass slides (Fisher Scientific). Polyclonal antibodies raised in rabbits against IBAT were obtained from a commercial source (Research Genetics), and purified as described.[16] Frozen tissue sections were air dried at room temperature and fixed in –20°C acetone for 15 minutes. Sections were washed in phosphate-buffered saline and incubated for 5 minutes with 5% goat serum in phosphate-buffered saline to reduce background staining. A 1:10 dilution of affinity-purified anti-IBAT antibody was applied for 3 hours. Sections were washed and incubated with a 1:100 dilution of a secondary goat antirabbit Cy3-IgG antibody (Jackson ImmunoResearch Laboratories) for 30 minutes, then washed in phosphate-buffered saline. Slides were mounted using an aqueous medium containing Hoechst 33258 dye as a nuclear stain (Sigma). The slides were evaluated for distribution of the fluorescence signal under a fluorescence microscope (Axioscope II, Zeiss) equipped with a digital imaging and an intensity-measuring program for fluorescence systems (Axiocam, Zeiss). Signal density was measured as fluorescent intensity over background as previously described.[16] Preimmune serum served as a negative control.

Surface area quantification

The relative surface area of the small intestine was quantified using fluorescence microscopy (Axioscope II, Zeiss) equipped with a digital imaging and intensity-measuring program for fluorescence systems (Axiocam, Zeiss). A surface area index ratio was calculated by measuring the mucosal length of five consecutive villi and dividing this parameter by the length of the base of the villi. This was done in five random sections in each animal. The surface area index ratio in the neoileum was compared with normal ileum and jejunum.

Quantification of IBAT mRNA

Small sections of intestinal tissues (100 to 250 mg) from wild type animals and seeded segments were used to prepare total RNA[17] using the RNeasy Extraction Kit (Qiagen) following the manufacturer's protocol. The total RNA was incubated with RQ1 DNase (Promega) and additionally purified by applying to RNeasy-Mini columns (Qiagen). One microgram of purified total RNA from each sample was reverse-transcribed with reverse transcriptase (Gibco) and the cDNA used as templates in subsequent polymerase chain reactions (PCRs). A

582-bp fragment from the coding region of rat-IBAT was amplified using the primer pair rIBAT 1: 5′ ATCTTCGTGGGCTTCCTCTGTCAG 3′ and rIBAT2: 5′ TTCCAAGGCAACTGTTCGGC 3′. A 618-bp fragment from the coding region of rat-glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as a normalizing control, was amplified with the primer pair rGAPDH1: 5′ ATGGGGTGATGCTGGTGCTGA 3′ and rGAPDH2: 5′ GAATGGGAGTTGCTGTTGAAG 3′ as described by Kim and associates.[18] We first determined the number of PCR cycles at which the two PCR products were accrued by linear increases from these cDNAs. To this end, we varied the number of PCR cycles from 20 to 40. Linear increases in all samples were observed with 31 PCR cycles for rIBAT and 21 cycles for rGADPH (data not shown). After running all the samples for these cycles, PCR products were electrophoresed in a 1.2% agarose gel in 0.5X Tris-borate EDTA buffer (44.6 mmol/L TRIS-Base, 44.5 mmol/L boric acid, 0.5 mmol/L EDTA, pH 8.3) and the rat-IBAT and rat-GAPDH bands quantified by gel densitometry on a Nucleotech 5000 densitometry unit (Nucleotech).

Analysis of fecal bile acids

A weighed representative sample of homogenized fecal material was mixed with an equal weight of distilled water in a glass tube with a Teflon-lined screw cap. To this was added chloroform/methanol (2:1 by volume), four volumes for each volume of water. The mixture was shaken thoroughly at intervals over a period of 1 hour, and then centrifuged at 1,200 *g* for 10 minutes. The upper layer (methanol/water) was carefully removed with a Pasteur pipette, filtered, and dried under nitrogen at 45°C.

A 300-mg solid-phase extraction cartridge (C18 Bond Elut, Varian) was washed with 10 mL of methanol, followed by 10 mL of distilled water. The dried residue from the fecal extract was dissolved in 3 mL of water and applied to the cartridge, which then was washed with 10 mL water and 3 mL of hexane. Bile acids were then eluted into a fresh tube with 3 mL of methanol. The latter was evaporated to dryness as described previously and taken up in 1 mL methanol for high pressure liquid chromatography analysis.

High pressure liquid chromatography analysis for conjugated and free bile acids was carried out with a three-pump Rainin Dynamax system (Rainin Instruments), by a modification of the method of Scalia,[19] in which ammonium acetate was substituted for the phosphate buffer, to use the greater sensitivity of the "mass detector." The sample was applied to a 4.6 × 250 mm Spherisorb ODS2, 5μ column (Phase Separations Ltd) and eluted with a gradient from 65% methanol/35% 0.03 M ammonium acetate, pH 4.5 to 90% methanol/10% ammonium acetate buffer, followed by 100% methanol. Detection was by an evaporative light-scattering detector (ELSD MK III, Alltech), which had been calibrated previously for quantitation with 14 standard bile acids.

Statistical methods

All data are expressed as mean ± SEM. Statistical analysis was performed using an analysis of variance and two-tailed *t*-test to compare individual groups as a posthoc test. A p value < 0.05 was considered significant.

RESULTS

Thirty adult Lewis rats underwent an initial laparotomy. Seventeen of these underwent the second operation; eight of these survived. The initial high mortality in this study was from clinical complications. It was not attributable to malabsorption or bile acid losses. Mortality likely resulted from EDTA toxicity or reperfusion injury in the first six rats. The length of bowel debrided was reduced from 30 cm to 15 cm, resulting in decreased mortality in the immediate postoperative period. Four additional rats died immediately after the second operation. The cause of death in the remaining animals included obstruction, intraperitoneal abscess, mesenteric torsion of the transplant segment, and devascularization of the transplant segment during the second operation. Two rats were sacrificed during the second operation because of an inability to separate structures adequately to restore intestinal continuity. Two rats died as a result of devascularization of the neoileum from excessive mobilization from dense adhesions. Repeat experiments were carried out on three additional rats without complication or mortality, as a result of modifications and refinements to this model. There was no mortality in any of the control groups.

The eight surviving animals in the treatment group underwent perfusion as described. But in one of the initial animals, the perfusion with EDTA solution was shortened to 20 minutes because of technical complications (animal 1). This led to incomplete mucosal de-

bridement. This animal went through the entire study period, but data gathered from this animal were not included in the treatment group.

Gross findings

Findings at the time of the second operation ranged from few film-like adhesions to dense adhesions with abscesses and fistula formation. Adhesiolysis was performed to mobilize the proximal neoileum. The neoileal segments varied in appearance from considerably dilated to shrunken in size. One of the eight rats that survived demonstrated a normal atrophic segment of bowel with few film-like adhesions. This rat (animal 1) had undergone incomplete debridement at the time of the initial operation. The degree of dilation and number of adhesions varied in the remaining rats that survived. All three rats in the repeat experimental group had minimal adhesions, a well-developed neoileal segment, and no evidence of abscess, fistula, or other inflammatory processes seen earlier in the development of this model.

Mucosal morphology

The neoileum was identified using antibodies to IBAT (Fig. 3). Neoileal mucosa was found in large patches within regenerated normal jejunal mucosa. The morphologic appearance of the neoileum was similar to that of native ileum. The surface index ratio was calculated by dividing the villous mucosal length by the length of the villous base. There was a trend toward increased surface area index ratio in the neoileum compared with the normal ileum (Fig. 4). The rat with inadequate debridement (animal 1) demonstrated a mucosal morphology that was similar to that of normal jejunum. There was no IBAT expression seen in the segment.

Bile acid loss in the stool

Bile acid loss in the stools was measured in positive controls ($n = 3$), negative controls ($n = 3$), preoperative controls ($n = 3$), and compared with the experimental group ($n = 8$) (Fig. 5). The bile acid profile in the stool of the positive control group was similar to that in the preoperative control group. Rats with an ileectomy had a considerably higher bile acid loss compared with preoperative and positive control groups ($p < 0.001$). The stool produced by rats after ileectomy was somewhat softer than normal rat feces, yet it maintained its form. Rats that had an ileectomy with anastomosis of neoileum in its place demonstrated a marked reduction in bile acid loss in the stool compared with rats with ileectomy alone ($p < 0.001$). The stool consistency in these animals with a neoileum was similar to the stool of normal preoperative controls.

Sodium-dependent and sodium-independent uptake of bile acids

To confirm findings of the fecal bile acid analysis, several segments containing native jejunum, ileum, and neoileal mucosa were examined for the presence of active, sodium-dependent bile acid uptake. Total uptake of the conjugated bile acid taurocholate in rats with the neoileal segment was notably higher than in the native jejunum ($p < 0.001$), yet less than that seen in the ileum (Fig. 6). Uptake by passive diffusion of bile acids in the neoileum was similar to that in the native ileum, which was approximately twofold greater than that found in the jejunum. The difference between total and sodium-independent taurocholate uptake represents the active, sodium-dependent absorption through IBAT. This active uptake was notably higher in the neoileum compared with jejunum ($p < 0.001$) (Fig. 6). Diffusional (sodium-independent) uptake was notably lower in the jejunum compared with both neoileum ($p < 0.001$) and ileum ($p < 0.001$).

IBAT protein and mRNA expression

Average IBAT protein expression in the group with a neoileum was notably greater than in the jejunum ($p < 0.001$), but was less than in the ileum (Fig. 7A). The rat with apparently incomplete debridement of the isolated jejunal segment demonstrated no IBAT protein expression.

We quantified the IBAT mRNA using reverse transcriptase PCR and gel densitometry. The IBAT signal intensity relative to GAPDH controls in the neoileum was similar to that in normal rat ileum, and was notably higher than in the jejunum ($p < 0.003$) (Fig. 7B). IBAT mRNA was not present in the rat with incomplete debridement.

DISCUSSION

This study is the first to show that intestinal stem cell transplantation can correct a clinical malabsorption syndrome. Previously, we successfully engrafted ileal stem cells into a demucosalized segment of jejunum, confirming the presence of a neoileum with antibodies to the

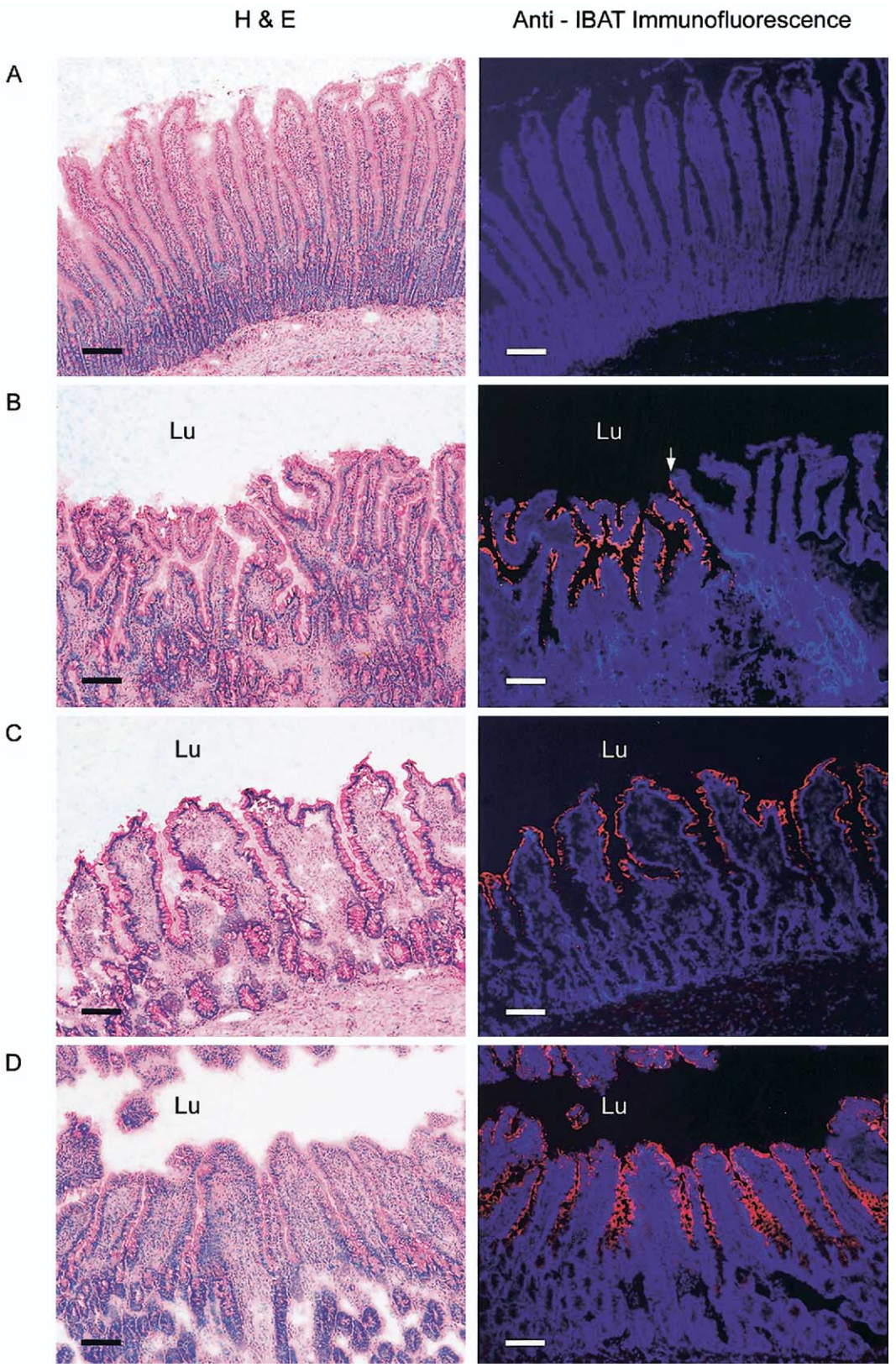

Figure 3. Hematoxylin and eosin (H&E) and immunofluorescence staining with anti-ileal bile acid transporter (IBAT) antibody (red = IBAT-positive). (A) native jejunum; (B) neoileum 4 wks after being anastomosed in place of resected ileum. In this section, the neoileal mucosa is located adjacent to native regenerated jejunal mucosa (neoileal mucosa [to left of arrow] and residual native jejunal mucosa [to right of arrow]). (C) neoileum; (D) native ileum. Scale bar 100 μm.

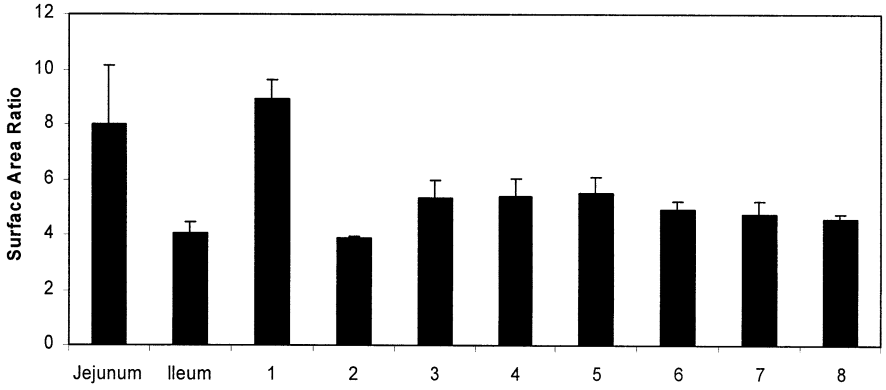

Figure 4. Villus mucosal length to villus base ratio. First two bars represent native jejunum and ileum. Numbers 1 to 8 on the x axis represent rats with neoileum anastomosed in place of resected ileum. Rat 1 underwent incomplete debridement resulting in restitution of native jejunum. Rats 2 to 8 demonstrated a ratio similar to native ileum.

IBAT protein.[9] In the present experiments, the neoileal segment was placed into continuity with the native small bowel after ileectomy, resulting in a reversal of bile acids losses in the stool. This data were corroborated with in vitro uptake studies, mucosal morphology, and IBAT mRNA and protein expression.

Measurement of bile acid excretion in the stool provided a physiologic means of assessing the in vivo functionality of the neoileum. The consistency of rat stool after an ileectomy was soft, yet it maintained its form, making this a less reliable clinical sign of bile acid loss. Rats with a neoileum anastomosed in place of a resected native ileum demonstrated a marked reduction of bile acid excretion compared with the rats undergoing ileectomy alone. There was a trend toward decreased bile acid excretion in both the rats with the neoileal segment and the positive controls (jejunectomy only) compared with normal preoperative controls. In both groups, rats underwent resection of a 30-cm segment of small bowel. Previous studies have demonstrated increased mucosal surface area in rodents undergoing bowel resection.[20] We found that neoileum had a surface area index ratio that was higher than that in normal ileum, suggesting mucosal hypertrophy may have played a role in the decreased excretion of stool bile acids in the experimental group.

Sodium-dependent bile acid absorption in the native ileum was 1.3-fold greater (57% of the total bile acid absorption) than sodium-independent bile acid absorption. Total bile acid absorption in the ileum was approximately fivefold greater than in the jejunum. Bile acid absorption in the jejunum resulted from sodium-independent uptake only. The neoileum demonstrated

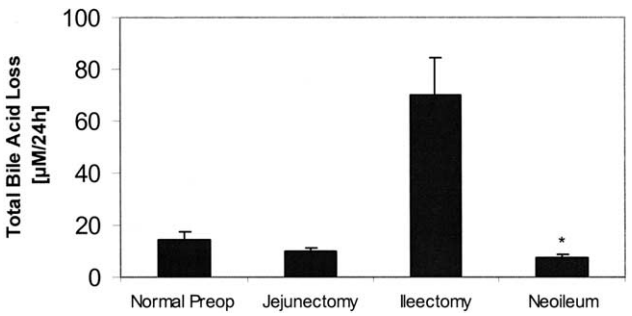

Figure 5. Quantification of total bile acid excretion in the stool of normal preoperative controls, positive controls (jejunectomy), negative controls (ileectomy) and the experimental group ie, the rats with neoileum anastomosed in place of the resected ileum. The experimental group demonstrated a notable reduction in bile acid excretion in the stool compared with negative controls to normal values (*$p < 0.001$ versus ileectomy group).

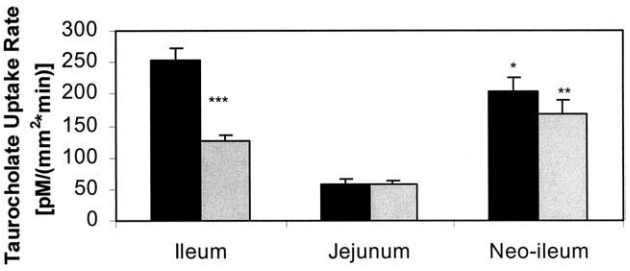

Figure 6. Bile acid uptake measurements in neoileal mucosa and normal gut. Total (black columns) and sodium-independent (gray columns) taurocholic acid uptake rates were measured in native jejunum, native ileum, and neoileum. Total taurocholate uptake in the neoileum was notably higher than that in the jejunum (*$p < 0.001$). The active sodium-dependent uptake rates (total minus sodium-independent) were notably higher in the neoileum compared with the jejunum (**$p < 0.001$) but lower than in ileum (***$p < 0.001$).

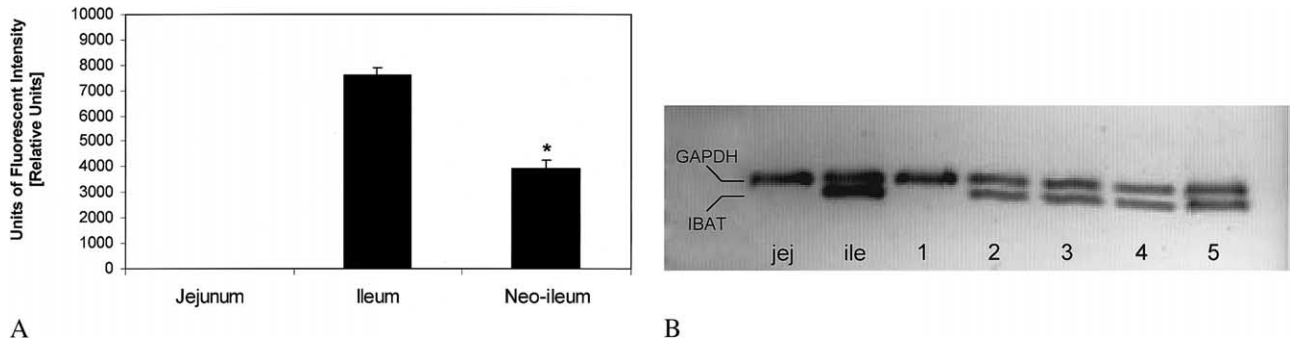

Figure 7. Expression of ileal bile acid transporter (IBAT) mRNA and IBAT protein. (A) IBAT protein expression is represented as units of fluorescence intensity in native jejunum, native ileum, and neoileum. The neoileum has significantly higher IBAT protein expression than the native jejunum (*$p < 0.001$). (B) Representative agarose gel of reverse transcription polymerase chain reaction cDNA products for glyceraldehyde-3-phosphate dehydrogenase (GADPH) and IBAT mRNA. Lane 1, jejunum; lane 2, ileum; lanes 3 to 7 represent the five rats in the experimental group (neoileum). Note: Lane 3, rat with incomplete debridement and reconstitution of jejunal mucosa. ile, ileum; jej, jejunum.

total bile acid uptake that was approximately fourfold greater than that in the jejunum, but the sodium-dependent component was only 33% of the total bile acid uptake as compared with 57% in ileal controls. From this, we concluded that sodium-dependent bile acid absorption in the neoileum was not quite as efficient as in the native ileum. There are a number of possibilities as to why this occurred.

First, our histologic examinations suggested that the mucosa of the neoileum is indeed a patchwork of areas featuring transplanted ileocytes and restituted native jejunum derived from crypts that were not completely debrided (Fig. 3B). That means the neoileum does not completely equal native ileal mucosa. Nonetheless, the neoileum appears a functionally adequate substitute for native ileum because its presence in the alimentary canal reversed bile acid malabsorption in our experiment. Second, the discrepancy between mRNA levels in the neoileum and IBAT protein levels may suggest that the neoileum does not express IBAT protein and native ileal mucosa. Additional studies are needed to examine this possibility. Third, the apparent increase in sodium-independent absorption may be from limitations inherent in the measurement methodology for everted sleeves. Calibration of this test is based on normal rat mucosa, which differs in its physical properties from neoileum. For example, the decreased compliance of the neoileal tissue may have resulted in greater stretching of the neoileal mucosa and, potentially, some microscopic disruption in the mucosa because it was fixed to the mounting rod during uptake measurements, resulting in increased passive absorption. Because of the paucity of available neoileal tissue, we were not able to rule out this possibility by histologic examination of the tissues used in the uptake study.

In summary, this study demonstrated that intestinal stem cell transplantation is capable of correcting a clinical intestinal malabsorption syndrome. These experiments provide proof of principle that intestinal stem cell transplantation is feasible and has substantial therapeutic potential. Because ileocytes may be viewed as jejunocytes with added features, ie, capabilities for active uptake of bile acids and vitamin B12, our study also offered a potential paradigm for intestinal gene therapy. One could envision future studies that aim at engrafting of genetically modified stem cells. So successful transplantation of enteric mucosal stem cells is a potentially important step in the development of intestinal stem cell gene therapy to treat a variety of gastrointestinal diseases.

Author Contributions

Study conception and design: Avansino, Stelzner
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