Stimulation of intestinal growth and function with DPP4 inhibition in a mouse short bowel syndrome model

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Sueyoshi R, Ignatoski KM, Okawada M, Hartmann B, Holst J, Teitelbaum DH. Stimulation of intestinal growth and function with DPP4 inhibition in a mouse short bowel syndrome model. Am J Physiol Gastrointest Liver Physiol 307: G410–G419, 2014. First published June 26, 2014; doi:10.1152/ajpgi.00363.2013.—Glucagon-like peptide-2 (GLP-2) has been shown to be effective in patients with short bowel syndrome (SBS), but it is rapidly inactivated by dipeptidyl peptidase IV (DPP4). We used an orally active DPP4 inhibitor (DPP4-I), MK-0626, to determine the efficacy of this approach to promote adaptation after SBS, determined optimal dosing, and identified further functional actions in a mouse model of SBS. Ten-week-old mice underwent a 50% proximal small bowel resection. Dose optimization was determined over a 3-day post-small bowel resection period. The established optimal dose was given for 7, 30, and 90 days and for 7 days followed by a 23-day washout period. Adaptive response was assessed by morphology, intestinal epithelial cell (IEC) proliferation (proliferating cell nuclear antigen), epithelial barrier function (transepithelial resistance), RT-PCR for intestinal transport proteins and GLP-2 receptor, IGF type 1 receptor, and GLP-2 plasma levels. Glucose-stimulated sodium transport was assessed for intestinal absorptive function. Seven days of DPP4-I treatment facilitated an increase in GLP-2 receptor levels, intestinal growth, and IEC proliferation. Treatment led to differential effects over time, with greater absorptive function at early time points and enhanced proliferation at later time points. Interestingly, adaptation continued in the group treated for 7 days followed by a 23-day washout. DPP4-I enhanced IEC proliferative action up to 90 days postresection, but this action seemed to peak by 30 days, as did GLP-2 plasma levels. Thus DPP4-I treatment may prove to be a viable option for accelerating intestinal adaptation with SBS.

Short bowel syndrome; dipeptidyl peptidase IV; sodium-glucose transport protein-1; intestinal epithelial cell; proliferation

SHORT BOWEL SYNDROME (SBS) is due to a loss of small intestinal length and has an incidence of 3–5 per 100,000 births per year (4). SBS is a devastating process with an associated reduced quality of life and significant complications (27). Current therapies include parenteral nutrition, novel surgical bowel-lengthening procedures, and intestinal transplantation. While essential to deliver needed nutrients, total parental nutrition can be highly morbid, leading to loss of vascular access, central catheter-associated infections, systemic infections, and associated liver disease (25). Intestinal transplantation requires lifelong immunosuppression, and surgical bowel lengthening has not been proven to be a long-term solution, with up to a 50% failure rate at 5 yr. Thus new treatments are needed for SBS.

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Glucagon-like peptide (GLP)-2 (GLP-2) is a peptide hormone with multiple beneficial effects on the intestine, including expansion of mucosal absorptive capacity, stimulation of intestinal epithelial cell (IEC) proliferation, and inhibition of IEC apoptosis, as well as enhancement of nutrient digestion, absorption, motility, and blood flow (22); recently, enhanced intestinal vascularization (28) has been seen with GLP-2. However, endogenous GLP-2 is rapidly (within 7 min) inactivated, predominantly by the endogenous serine protease dipeptidyl peptidase IV (DPP4) (5). To address this fast degradation, GLP-2 mimetics, such as teduglutide, that are resistant to DPP4 cleavage have been developed. Recently, DPP4-resistant GLP-2 analogs, such as teduglutide, have been subjected to clinical trials. These trials demonstrate that GLP-2 is safe and well-tolerated and promotes intestinal growth in adult SBS patients (16, 19). However, there are some important disadvantages to the use of DPP4-resistant GLP-2 analogs. The growth-promoting effects of GLP-2 in mice and humans and the effects of teduglutide on nutrient absorption in humans with SBS can be reversed upon withdrawal of treatment (3, 16, 31). Thus teduglutide may well require chronic administration, and potential long-term adverse effects cannot be discounted (30). Additionally, the cost of maintaining patients on teduglutide is nearly $300,000 per year (14, 21), and daily injection of the drug is required. Therefore, other strategies to optimize intestinal growth could involve the use of the organism’s endogenous GLP-2 production. We previously showed that DPP4 inhibitor (DPP4-I) was efficacious for SBS in a mouse model (20). Short-term (3 days) administration of the inhibitor stimulated cell proliferation and morphological changes. However, it is unknown how DPP4-I affects intestinal function in an animal surgical model. It has also not been determined whether the efficacy of administration of DPP4-I is persistent over the long term in a SBS model.

MK-0626 is an orally active DPP4-I that is similar to the widely used clinical agent sitagliptin for diabetic therapy. Additionally, MK-0626 is more selective and has a higher bioavailability than the DPP4-I we used previously. Thus we hypothesized that MK-0626 would be more efficacious in our murine SBS model than was previously observed (6). In this study we also determined the optimal dose of this DPP4-I during a short-term study and, finally, investigated the chronic effects of the drug by using an optimized dose in long-term studies. The results of this study demonstrate a potential future clinical use for this approach in many patients with SBS.

MATERIALS AND METHODS

Experimental animals. All animal experiments were conducted with approval from the University of Michigan Committee on the Use
and Care of Animals (protocol no. 07703/03986). Specific pathogen-free 10-wk-old C57BL/6 male mice (>22.0 g body wt; Jackson Laboratory, Bar Harbor, ME) were maintained in a 12-h night rhythm at 23°C and a relative humidity of 40–60%. Animals were fed a standard rodent diet (LabDiet 5001 Rodent Diet, PMI Nutrition International, Brentwood, MO) ad libitum. At 48 h before surgery, the diet was changed to a microstabilized rodent liquid diet (TestDiet, Purina, Richmond, IN), which was continued for 1 mo after SBS creation.

Drug treatment. MK-0626 (kindly provided by Merck Sharp & Dohme, Whitehouse Station, NJ), an orally active DPP4-I, was given by oral gavage once or twice daily (at 12-h intervals) at 1 or 3 mg/kg for 3 days to determine the optimal dose for longer-term experiments. The drug was then given at 3 mg/kg for 7, 30, and 90 days twice daily. DPP4-I was given by gavage to ensure matched dosing and timing of drug intake in all groups.

Surgical procedure. Anesthesia was induced and maintained by inhalational administration of isoflurane. Buprenorphine was injected into the subcutaneous space pre- and postoperatively for pain control. The SBS model consisted of a resection of small bowel starting 5–8 cm distal to the ligament of Treitz and ending 7–10 cm proximal to the ileocecal valve; it was followed by an end-to-end jejunoileal anastomosis with 9-0 nylon suture, similar to that previously described (12, 20). Normal saline (1.5 ml) was injected into the peritoneal cavity before closure of the small bowel. Peritoneum and skin were closed using 4-0 polyglactin sutures (see schematic of the surgical procedure in Fig. 1).

Collection of tissue. For the experimental period, the mice were maintained on a microstabilized rodent liquid diet (TestDiet) and 5% dextrose water (dextrose water only was provided on days 1–3 postsurgery to ensure some nutritional intake during the immediate postoperative period). DPP4-I was given twice daily for 3, 7, 30, or 90 days. The mice were euthanized on postoperative days 1–3, or 90 days twice daily. DPP4-I was given by gavage to ensure matched dosing and timing of drug intake in all groups.

Barrier function and glucose transport assessment. Briefly, an Ussing chamber was utilized with freshly isolated small intestine tissue according to standard techniques (33). Intestinal tissues with an exposed surface area of 0.031 cm² were incubated in 5 ml of preheated 37°C Krebs buffer (in mM: 140 NaCl, 1.2 MgCl₂, 1.2 CaCl₂, 10 KHCO₃, 0.2 KH₂PO₄, and 1.2 K₂HPO₄) on each side of the Ussing chamber, or mucosal tissue was scraped for protein assay and RT-PCR. Images were visualized on a Nikon Eclipse Ti microscope under ×20 magnification; 15 crypts per slide were analyzed.

RT-PCR. Scraped intestinal mucosal tissue was added to the RNeasy Micro Kit (Qiagen, Hilden, Germany) and homogenized. cDNA was purified and processed as previously described (24). RT-PCR was performed for glucose transporters using a real-time analyzer (Rotor-Gene 6000, Qiagen). All primers for selected gene sequences were suggested by Primer-BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and are listed in Table 1. β-Actin was used as an internal control for all quantitative analyses of mRNA expression.

Table 1. Primer design

<table>
<thead>
<tr>
<th>Primer</th>
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<tr>
<td>SGLT1 Forward</td>
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<tr>
<td>SGLT1 Reverse</td>
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<tr>
<td>GLUT2 Forward</td>
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<tr>
<td>GLUT2 Reverse</td>
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<tr>
<td>GLUT5 Forward</td>
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<tr>
<td>GLUT5 Reverse</td>
</tr>
<tr>
<td>GLP-2R Forward</td>
</tr>
<tr>
<td>GLP-2R Reverse</td>
</tr>
<tr>
<td>IGF-1 Forward</td>
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<tr>
<td>IGF-1 Reverse</td>
</tr>
<tr>
<td>IGF-1β Actin Forward</td>
</tr>
<tr>
<td>IGF-1β Actin Reverse</td>
</tr>
<tr>
<td>TGTCCGAGGTCAGCCTGAGG</td>
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<td>TGTCCGAGGTCAGCCTGAGG</td>
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SGLT1, sodium-glucose transporter-1; GLUT2 and GLUT5, glucose transporters; GLP-2R, glucagon-like peptide-2 receptor; IGF-1R, IGF-1 receptor.
since 1985 as an indication of net active ion transport. Then the change in

Blood sugar level, serum amylase, and plasma GLP-2 level. Since DPP4-I is used to treat type 2 diabetes mellitus, it is possible that glucose levels might decline with its use. Therefore, we investigated blood glucose levels on postoperative days 3, 7, 14, 21, 30, and 90. We detected blood sugar levels using OneTouch Ultra (Lifescan, Milpitas, CA) 2 h after drug administration. In addition, DPP4-I has been shown to cause pancreatitis in some settings; thus we also examined serum amylase levels and plasma GLP-2 at the end of the study. Blood was collected postmortem. The blood samples for amylose were centrifuged at 8,000 rpm for 10 min in 4°C, and only serum was collected and tested. Plasma samples for GLP-2 were processed as previously described (20). Plasma GLP-2 was measured

PCNA / DAPI

![Image](http://ajpgi.physiology.org/)

Fig. 2. Intestinal epithelial cell (IEC) proliferation in jejunum and ileum. IEC proliferation was measured by immunofluorescence staining for proliferating cell nuclear antigen (PCNA). DPP4-I (3 mg/kg every 12 h) led to a significant increase in IEC proliferation vs. placebo groups at all time points in jejunum and ileum. Placebo 30-day group in jejunum and placebo 3-day and 30-day groups in ileum also exhibited a marked increase in cell proliferation compared with naive group. DAPI, 4′,6-diamidino-2-phenylindole. Values are means ± SE; n = 5 or 6 per group. *P < 0.05 vs. naive; †P < 0.05 vs. corresponding placebo (by ANOVA).
by radioimmunoassay using an antibody specific to the NH₂ terminus of GLP-2. Results are expressed as picomoles per liter (10, 17).

**Protein (Western) blotting.** Scraped intestinal mucosal tissues were lysed in RIPA buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 1% Igepal, 0.25% sodium deoxycholate, 10 μg/ml aprotinin, and 5 μl/ml leupeptin). After homogenization and centrifugation at 14,000 g for 10 min at 4°C, the supernatant was stored in Laemmli loading dye. Equal amounts of protein were separated on 4–20% SDS-polyacrylamide gels (Bio-Rad, Hercules, CA) and then transferred to a polyvinylidene difluoride membrane. Blots were blocked in 5% BSA and washed with Tris-buffered saline containing 0.05% Tween (TBS-T). The membranes were incubated for 1 h with secondary antibody. After two additional washes with TBS-T, the membranes were developed for fluorescence reagent using the manufacturer’s protocol (SuperSignal West Femto, Thermo Fisher Scientific, Logan, UT).

**Statistics.** At least five animals were used per group per study, as determined by power analysis. Values are means ± SE. For comparison of two groups, unpaired t-tests were used, and ANOVA with Bonferroni’s post hoc test was used to determine the significance of differences when more than one group was compared. Each study group was compared with the naive group and with the placebo group for the same time point. Differences between groups were tested using pair-wise comparisons within the model. P < 0.05 was considered statistically significant.

**RESULTS**

**Determination of the optimal dose of DPP4-I in a mouse SBS model.** Because this particular DPP4-I, MK-0626, had not been used for treatment of SBS, dose optimization was performed over a short (3-day) time frame of administration, a duration chosen because of our preliminary data with another DPP4-I (20). On the basis of their previous data, Merck Sharp & Dohme, which provided MK-0626, suggested doses of 1 and 3 mg/kg. We distributed mice into five groups: control (PBS only), 1 mg/kg once daily, 1 mg/kg twice daily (at 12-h intervals), 3 mg/kg once daily, and 3 mg/kg twice daily over 3 days. Body weight changes and blood glucose levels were not statistically significant between groups. All groups treated with DPP4-I had significantly increased IEC proliferation compared with the placebo group in the jejunum and ileum, except the 1 mg/kg once daily group in the jejunum (P < 0.05; Table 2). Body weight declined, which is typical after a major intestinal resection, but no group showed a >18% decline from original weight.

We examined the morphology of the mucosa in all groups. The 1 mg/kg once daily group in the jejunum, along with the 1 mg/kg once daily and 3 mg/kg once daily groups in the ileum...
exhibited statistically significant increases in crypt depth compared with each placebo group ($P < 0.05$; Table 2). No statistically significant change in villus height was observed. Plasma GLP-2 levels were statistically increased (~2-fold) in all groups and achieved significant elevation in the 1 mg/kg once daily and 3 mg/kg once daily groups compared with placebo ($P < 0.05$; Table 2). We determined the efficiency of the glucose transport in the jejunum and ileum as a marker of intestinal function (Table 2). Glucose transport in the jejunum and ileum was significantly increased in the 3 mg/kg twice daily group ($P < 0.05$). On the basis of IEC proliferation and intestinal function, we selected 3 mg/kg twice daily as the optimal dose for the subsequent studies. In the remainder of the experiments, we administered 3 mg/kg twice daily for 7, 30, and 90 days. Since we observed effects at 3 and 7 days that were similar to effects at 30 days, we treated an additional group of SBS mice for 7 days followed by 23 days of DPP4-I washout (DPP4-I 7-day/PBS 23-day group).

**DPP4-I** stimulated epithelial cell proliferation in the jejunum and ileum at all time points. PCNA staining was performed to determine IEC proliferation at each time point. At all time points, the SBS group showed increased IEC proliferation compared with naive (nonoperated) mice. DPP4-I significantly stimulated IEC proliferation beyond this adaptive state compared with most placebo groups in the jejunum and ileum ($P < 0.05$; Fig. 2, Table 3). While the dominant action of the drug was observed in the jejunum, where GLP-2 receptor density is greater, DPP4-I also quite effectively promoted ileal IEC proliferation.

**Fig. 4.** DPP4-I promoted intestinal function in the early phase. A: change in isoelectric (short-circuit) current ($I_{sc}$) was determined in Ussing chambers by mucosal application of glucose and used as a functional measure of glucose transport. $I_{sc}$ changes were statistically upregulated in jejunum and ileum in DPP4-I 3-day group. # $P < 0.05$ vs. corresponding placebo. B–D: abundance of nutrient transporters (sodium-glucose transporter (SGLT-1) and glucose transporters (GLUT2 and GLUT5)) at the RNA level was measured using RT-PCR. Expression of SGLT-1 and GLUT2 mRNA was significantly upregulated in DPP4-I 3-day group compared with placebo 3-day group. There was no effect on GLUT5 expression. These results may indicate that DPP4-I affects intestinal function in the early phase. Values are means ± SE; n = 6 specimens per group. *$P < 0.05$ vs. corresponding placebo (by ANOVA).
proliferation at all time points except 90 days, when proliferation levels were similar between placebo and treatment groups.

**DPP4-I treatment led to greater morphological adaptive changes.** Intestinal morphology was examined in each group (Fig. 3, Table 3). As with proliferation, the performance of a SBS model led to an independent adaptive state, resulting in some increase in villus height but a predominant increase in crypt depth. DPP4-I led to an incremental increase in crypt depth at every time point in the jejunal specimens and at 3, 7, and 30 days and in the DPP4-I 7-day/PBS 23-day group in the ileum. Administration of DPP4-I for 7 days had the greatest effect in the jejunum: villus height = 454.0 ± 16.1 and 404.1 ± 13.2 μm in the DPP4-I 7-day and placebo 7-day groups, respectively (P < 0.05); crypt depth = 135.8 ± 5.2 and 106.1 ± 2.8 μm in the DPP4-I 7-day and placebo 7-day groups, respectively (P < 0.001). In the DPP4-I 7-day/PBS 23-day group, villus height increased 25% in the jejunum, and although this increase was not significantly different from the other 7-day groups, it represented the largest increase among the groups. Although adaptation was somewhat less, the increase in crypt depth in the ileum was statistically significant in the DPP4-I 7-day (P < 0.05), DPP4-I 30-day (P < 0.001), and DPP4-I 7-day/PBS 23-day (P < 0.01) groups.

**DPP4-I promoted intestinal functional changes at an early time point after SBS formation.** Glucose transport is a functional marker of the intestine, and glucose uptake was examined in full-thickness intestinal segments mounted in Ussing chambers. Transport was detected by the relative current change upon addition of glucose to the mucosal side of the tissue. Interestingly, glucose transport changes were significantly upregulated in the jejunum and ileum in the DPP4-I 3-day group compared with the placebo 3-day group (P < 0.05; Fig. 4A). I<sub>e</sub> changes were not significantly different at the other time points.

As an additional approach to examining functional changes, a selected group of transporters that would reflect intestinal glucose transport were examined at the mRNA level. In the ileum, mRNA abundance of SGLT1 and GLUT2 (Fig. 4, B and C) was significantly upregulated in the DPP4-I 3-day group compared with the placebo 3-day group (P < 0.05). Significant changes were not detected in the jejunal specimens. While a trend toward increased GLUT5 expression was noted in jejunal and ileal specimens over time, no significant differences between treatment and placebo groups were detected (Fig. 4D).

Next we used Western blotting techniques to confirm the upregulation of SGLT1 protein (Fig. 5). SGLT1 expression was markedly increased in the DPP4-I 3-day group vs. placebo

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**Table 4. Effects of DPP4-I on GLP-2R and IGF-1**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>GLP-2R</th>
<th>IGF-1</th>
<th>IGR-1R</th>
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<tr>
<td></td>
<td></td>
<td>Jejunum</td>
<td>Ileum</td>
<td>Jejunum</td>
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<tr>
<td>Naive</td>
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<td>6.9 ± 2.6</td>
<td>4.6 ± 0.5</td>
<td>8.5 ± 3.0</td>
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<tr>
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<td>25.7 ± 18.4</td>
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<tr>
<td>DPP4-I 3-day</td>
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<td>7.1 ± 1.9</td>
<td>11.5 ± 4.0</td>
<td>37.5 ± 19.2</td>
</tr>
<tr>
<td>Placebo 7-day</td>
<td>6</td>
<td>12.0 ± 3.1</td>
<td>4.0 ± 1.2</td>
<td>18.0 ± 11.4</td>
</tr>
<tr>
<td>DPP4-I 7-day</td>
<td>6</td>
<td>30.1 ± 5.9&lt;sup&gt;±&lt;/sup&gt;</td>
<td>5.5 ± 2.0</td>
<td>64.1 ± 21.4&lt;sup&gt;±&lt;/sup&gt;</td>
</tr>
<tr>
<td>Placebo 30-day</td>
<td>5</td>
<td>7.5 ± 1.6</td>
<td>5.4 ± 1.5</td>
<td>9.0 ± 1.8</td>
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<tr>
<td>DPP4-I 30-day</td>
<td>7</td>
<td>15.8 ± 6.1</td>
<td>4.2 ± 1.3</td>
<td>11.4 ± 5.0</td>
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<tr>
<td>DPP4-I 7-day/PBS 23-day</td>
<td>6</td>
<td>72.9 ± 15.8&lt;sup&gt;±&lt;/sup&gt;</td>
<td>20.0 ± 7.8</td>
<td>118.1 ± 34.4&lt;sup&gt;±&lt;/sup&gt;</td>
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Values are means ± SE relative to β-actin. *P < 0.05 vs. naive group. †P < 0.05 vs. placebo group.
for the same time point ($P < 0.05$). However, levels of SGLT-1 were not significantly different from naive controls. It is possible that SGLT1 may normally be downregulated by surgical resection of the small bowel (placebo group) but is upregulated by a DPP4-I. These results suggest that DPP4-I affects intestinal function at a very early time point after intestinal resection (3 days) and that this effect was not sustained at later time points.

**DPP4-I drove an increase in GLP-2 plasma levels and GLP-2 receptor and IGF-1 receptor mRNA expression.** We next determined GLP-2 receptor (GLP-2R) and IGF-1 receptor (IGF-1R) mRNA expression by RT-PCR in each study group (Table 4) to examine for a potential feedback response of blocking DPP4 and potential mechanisms that may be promoting this action above the increase in GLP-2. Concordant with the histological results, GLP-2R expression in the jejunum ($P < 0.01$) was statistically increased in the DPP4-I 7-day ($P < 0.05$) and DPP4-I 7-day/PBS 23-day ($P < 0.01$) groups compared with the respective placebo groups. In addition, the DPP4-I 7-day/PBS 23-day group exhibited markedly increased IGF-1 and IGF-1R mRNA expression in the jejunum compared with the placebo 30-day group. As well, 3-day and 30-day values were not significantly different from controls. These results suggest 7 days of an early drive toward absorption with this drug but continued proliferative effects beyond this time point.

Figure 6 shows serial plasma levels of GLP-2 as measured by radioimmunoassay. It was interesting to note that placebo- and DPP4-I-treated groups showed an increase in GLP-2 levels compared with naive controls and that this trend continued for the first 30 days. Although not completely significant, the DPP4-I-treated groups showed higher GLP-2 levels over this time period, and this trended to significance by day 30. Interestingly, despite improved adaptation, this increased GLP-2 was not seen in the DPP4-I 7-day/placebo 23-day group; potentially, this increased GLP-2 time period, and this trended to significance by 30 days. *

**Gross morphological changes and intestinal TER.** We next examined intestinal adaptation on a gross morphological basis and the effect of DPP4-I on epithelial barrier function (9). We calculated the intestinal diameter and circumference of transverse histological sections. Diameters were not different from naive mouse jejunum or ileum until 30 days. The DPP4-I 30-day group exhibited a significantly increased diameter and circumference compared with the placebo 30-day group (Fig. 7, A–C). The DPP4-I 7-day/PBS 23-day group also exhibited a significant increase in maximal diameter and circumference ($P < 0.05$). Diameter remained at this level at 90 days, and DPP4-I failed to drive further change compared with placebo.

TER in the jejunum was significantly increased in DPP4-I 30-day compared with placebo-treated mice ($P < 0.05$; Fig. 7, D and E), whereas TER was lower in the DPP4-I 7-day/PBS 23-day group. TER declined in the jejunal specimens from placebo-treated mice at 90 days, and this was partially prevented by administration of DPP4-I. TER in ileal specimens was significantly decreased in most of the treatment groups compared with the placebo-treated groups. Potentially, this loss of TER may represent a rapid migration of IEC along the crypt-villus axis, leading to a transient breakdown of barrier function.

**Assessment of body weight and blood glucose with DPP4-I.** Because the class of DPP4-I drugs is used to treat type 2 diabetes, we measured body weight and blood glucose levels weekly (Table 5). Body weights in the DPP4-I- and placebo-treated groups were not significantly different up to day 90. Interestingly, at day 90, the DPP4-I-treated group showed significant weight loss compared with the placebo-treated group.

We examined the glucose level in blood obtained weekly from the tail vein. At postoperative day 14, glucose levels were markedly reduced in the DPP4-I-treated group compared with the placebo-treated group (153.3 ± 9.6 vs. 190.0 ± 13.1 mg/dl, $P < 0.05$); however, glucose levels remained within a normal range. Glucose levels at other time points were not statistically different (Table 5).

**Long-term administration of DPP4-I did not lead to chemical evidence of pancreatitis.** Because DPP4-I has been linked to pancreatitis when used as a type 2 diabetes drug (32), we examined serum amylase levels. Serum amylase levels remained within the normal range at all time points (Table 5).

**DISCUSSION**

SBS is the loss of significant small intestinal length, resulting in an inability to enterally absorb sufficient nutrients and electrolytes essential for survival. SBS is associated with significant complications and a reduced quality of life (27). Patient outcomes have been linked to the length and function of remaining small intestine (25); therefore, efficacious, cost-effective therapies to increase intestinal mass are greatly needed. GLP-2 enhances glucose and nutrient uptake, which contributes to repair of intestinal damage (23) and has various beneficial effects in a SBS model (22), indicating that long-lasting increases in GLP-2 may be beneficial in the treatment of SBS. However, long-term GLP-2 treatment is very expensive,
exceeding $300,000 per year, and requires daily injection. To circumvent the cost of long-term GLP-2, we investigated a novel approach with a DPP4-I. On average, the yearly cost of this class of drugs is well under $1,500; thus substantial savings may accrue with its use. DPP4 cleaves GLP-2 in 7 min in the serum; thus, blocking DPP4 activity would increase endogenous GLP-2. We previously examined the efficacy of short-term inhibition of the DPP4-I sitagliptin phosphate in repair of a SBS model of mouse intestine (20). DPP4 inhibition enhanced endogenous GLP-2 upon administration of a liquid diet in mice (11). Concordant with GLP-2 facilitating repair of the intestine, we found that 3 days of DPP4 inhibition increased plasma GLP-2 levels, cell proliferation, and villus height and crypt depth. We hypothesized that a more prolonged course with DPP4-I would result in greater adaptation. We also wanted to test the most optimal time and dosing of such a drug, as it is believed that findings from this approach may have strong translatable applications to the treatment of SBS. For the present study we used MK-0626, which is more selective and has better bioavailability than sitagliptin in animal studies (6) and may have adaptive action in our 50% proximal small bowel resection mouse model similar to sitagliptin. We used MK-0626 over 7, 30, or 90 days to assess whether better repair could be achieved with longer treatments after establishing an effective dose at 3 days. Additionally, we tested the duration of this treatment by examining adaptation 23 days after withdrawal of a 7-day course of MK-0626. We demonstrated that MK-0626 administration could stimulate cell proliferation, induce morphological change, increase glucose uptake, facilitate intestinal adaptation, and increase TER, implying that MK-0626 may be an effective therapy for SBS. Direct comparisons between clinical trials of SBS patients given teduglutide and the present rodent study are difficult, in that clinical trials only reported on crypt/villus morphology and did not report proliferation data. However, it was interesting to note that crypt depth in human trials increased from 17% to 64% over a 24-wk period (15). Furthermore, in a rat model of SBS, crypt cell numbers (depth not reported) increased by ~20–24% over the study period in the group treated with exogenous human GLP-2 (17). Thus the contribution of exogenous GLP-2 was somewhat superior to the gains in our study of ~15% compared with placebo-treated mice. This is not entirely surprising, but future studies involving a more head-to-head comparison of these two treatment options for SBS are needed.

Swietlicki et al. (29) and Garrison et al. (9) showed that exogenous GLP-2 and GLP-2 analogs facilitate intestinal ad-
We also observed increases in nutrient transporter proteins by 7 days, and, quite importantly, this effect persisted through 23 days without additional DPP4-I treatment. In this same time frame, we observed that the TER for treated intestines was at its highest. Concordant with our other data, these data also indicate that adaptation occurs early and lasts for a considerable time after treatment has ended.

As the class of DPP4-I drugs have been approved by the US Food and Drug Administration for treatment of diabetes and this treatment enhances circulating GLP-1 levels, we were concerned about blood glucose levels. DPP4 inactivates not only GLP-2, but also GLP-1 and gastric inhibitory polypeptide. GLP-1 and gastric inhibitory polypeptide delay gastric emptying (13), which may reduce food intake (1), making DPP4-I weight-neutral or leading to weight loss (32). In fact, in the postoperative 90-day period, body weight levels were lower in the DPP4-I than placebo-treated group. However, glucose levels were not significantly different from placebo-treated groups at all time points. Despite this degree of safety, it will be important to closely monitor patients receiving these inhibitors. As well, DPP4-I can stimulate pancreatic secretion; thus there will be a critical need to monitor amylase and lipase levels. Again, amylase levels were consistently within or below the normal range for mice in the present study. One potential exception to the clinical use of DPP4-I in SBS will be individuals who have lost a substantial portion of the distal ileum and right colon, the predominant source of L cells, which produce GLP-2 (7). It is conceivable that even low levels of GLP-2 in these patients may be enhanced or that the use of these inhibitors may benefit SBS patients who might otherwise receive exogenous GLP-2 derivatives.

In conclusion, our results indicate that use of DPP4-I for treatment of SBS results in long-term adaptation benefits. The predominant benefit of optimal nutrient transport seems to occur early, but proliferative effects are seen with up to 3 mo of therapy. The treatment may be somewhat long-lasting, as a relatively short DPP4-I treatment time of 7 days followed by treatment withdrawal led to sustained efficacy. While restarting the treatment after this rest period may provide further adaptation, this approach will need to be addressed in future studies. It is hoped that these studies may prompt future clinical trials of the use of these agents for patients with SBS.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.S. and B.H. performed the experiments; R.S., B.H., and J.J.H. analyzed the data; R.S., K.M.W.I., M.O., B.H., J.J.H., and D.H.T. interpreted the results of the experiments; R.S. prepared the figures; R.S. drafted the manuscript; R.S., K.M.W.I., M.O., B.H., J.J.H., and D.H.T. approved the final version of the manuscript; K.M.W.I., J.J.H., and D.H.T. edited and revised the manuscript; M.O. and D.H.T. are responsible for conception and design of the research.
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