Stimulation of intestinal growth and function with DPP-IV inhibition in a mouse short bowel syndrome model

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Abstract

Glucagon-like peptide-2 (GLP-2) has been shown to be effective in patients with short bowel syndrome (SBS), but 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 SBS mouse model. Ten week old mice underwent a 50% proximal small bowel resection (SBR). Dose optimization was determined over a 3-day post-SBS period. The established optimal dose was given for 7 days, 30 days, 90 days, and 7 days followed by 23 days washout period. Adaptive response was assessed by morphology, intestinal epithelial cell (IEC) proliferation (PCNA), epithelial barrier function (transepithelial resistance), RT-PCR for intestinal transport proteins, GLP-2R, and IGF-1R, 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-2R levels, intestinal growth, and IEC proliferation. Treatment led to differential effects over time with greater absorptive function early, and enhanced proliferation at later time points. Interestingly, 7 day treatment followed by 23 days of non-treatment showed continued adaptation. DPP-IV-I enhanced IEC proliferative action up to 90-days post-resection, but this action seemed to peak by 30 days, as did GLP-2 plasma levels. Thus, use of DPP4-I treatment may prove to be a viable treatment for accelerating intestinal adaptation with SBS.

Keywords

Short bowel syndrome, dipeptidyl peptidase-IV (DPP4), SGLT1, intestinal epithelial cell, proliferation
Introduction

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 (5). SBS is a devastating process with an associated reduced quality of life and significant complications (30). Current therapies include parenteral nutrition, novel surgical bowel lengthening procedures and intestinal transplantation. While essential to deliver needed nutrients, total parental nutrition (TPN) can be highly morbid, leading to loss of vascular access, central catheter associated infections, systemic infections and associated liver disease (28). 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 years. Thus new treatments are needed for SBS.

Glucagon-like peptide-2 (GLP-2) is a peptide hormone with multiple beneficial effects on the intestine, including expansion on mucosal absorptive capacity, stimulation of intestinal epithelial cell (IEC) proliferation, inhibition of IEC apoptosis, as well as enhancement of nutrient digestion, absorption, motility and blood flow (25); and recently enhanced intestinal vascularization (32) has been seen with GLP-2. However, endogenous GLP-2 is rapidly inactivated (within 7 mins) predominately by the endogenous serine protease dipeptidyl peptidase-IV (DPP4) (6). To address this fast degradation, GLP-2 mimetics, such as teduglutide that are resistant to DPP4 cleavage, have been developed. Recently, DPP-IV-resistant GLP-2 analogs, such as teduglutide, have been subjected to clinical trials. These trials demonstrate that GLP-2 is safe, well-tolerated, and promotes intestinal growth in adult SBS patients (12, 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 those of teduglutide, on nutrient absorption in humans with SBS can be reversed upon withdrawal of treatment (4, 19, 35). Thus, teduglutide may well require chronic administration, and potential long-term adverse effects cannot be discounted (34). Additionally, the cost of maintaining patients on teduglutide is approximately 300,000 U.S. dollars per year (2, 17); and teduglutide requires a daily injection of the drug. Therefore, other strategies to optimize intestinal growth could come from the use of the organism’s endogenous GLP-2 production. We have previously shown that DPP4 inhibitor (DPP4-I) was efficacious for SBS in a mouse model (23). Short term administration of the inhibitor (3 days) stimulated cell proliferation and morphological changes. However it is still 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 previously observed (7). 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 usage of this approach for 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 number 07703/03986). Specific pathogen-free 10-week-old C57BL/6
male mice, with body weight over 22.0g (Jackson Bar Harbor, MA) were maintained in a 12 hr night rhythm at
23°C and a relative humidity of 40-60%. Animals were fed a standard rodent diet (LabDiet 5001Rodent Diet,
PMI Nutrition International, LLC, Brentwood, MO) ad libitum. Forty-eight hours before surgery, the diet was
changed to micro-stabilized rodent liquid diet (TestDiet, Richmond, IN). This liquid diet was continued for the
first month after SBS creation.

Drug Treatment

MK-0626 is an orally active DPP-IV-I and was given by oral gavage either once or twice daily (at 12
hour intervals) at either 1 mg/kg or 3 mg/kg for 3 days to determine the optimal dosage to use in longer-term
experiments. The drug was then given at 3 mg/kg for 7, 30, and 90 days twice daily. MK-0626 was kindly
provided by Merck Sharp and Dohme Pharmaceuticals (Whitehouse Station, NJ). The DPP4-I was given by
gavage to insure 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 subcutaneous space pre-operatively and post-operative 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, and it was followed by an end-to-end jejuno-ileal anastomosis with 9–0 nylon suture, similar to
that previously described(15, 24). Normal saline (1.5 ml) was injected to the peritoneal cavity before closing.
Peritoneum and skin were closed using 4-0 polyglactin sutures. See FIGURE 1 for a schematic of the surgical
procedure.

Collection of tissue

For the experimental period, the mice were maintained on a micro-stabilized rodent liquid diet (TestDiet,
Richmond, IN) and 5% dextrose water (dextrose water only for the first 3 days post- surgery, to insure some
nutritional intake during the immediate post-operative period). DPP4-I was given B.I.D. for 3 days, 7 days, 30
days, or 90 days. The mice were euthanized on post-operative days 3, 7, 30, or 90, depending on the experiment. Two 0.5 cm portions of the segments were excised (FIGURE 1). One was placed into 10% formaldehyde. Formalin-preserved sections were mounted in paraffin, sectioned transversely (5 µm), and stained with hematoxylin and eosin. Adjacent paraffin sections were later used for immunofluorescence staining. The remaining segment was immediately placed into Ussing chambers or mucosal tissue was scraped for protein assay and RT-PCR, as described previously (27).

**Intestinal morphology and epithelial cell proliferation (PCNA staining)**

The hematoxylin-eosin stained histological sections were visualized on a Nikon Eclipse TS100 (*Nikon Instruments Inc*, Tokyo, Japan) and analyzed the Image J software in a blinded manner. Villus height, crypt depth and intestinal perimeter were measured in each time period. Cell proliferation was evaluated with immunofluorescence staining using PCNA antibody (*Cell Signaling*, Danvers, MA), which was applied overnight (dilution- 1:1000). Slides were incubated with secondary antibody for 2 hrs and counterstained with DAPI for detection of nuclei. Cell proliferation rate was recorded as the number of PCNA positive crypt cells per the total number of crypt cells, as previously described (9).

Images were visualized on a Nikon Eclipse Ti (*Nikon Instruments Inc*, Tokyo, Japan) under 20X magnification. 15 crypts per slide were analyzed.

**Real Time-Polymerase Chain Reaction PCR (RT-PCR)**

Scraped intestinal mucosal tissue was added into RNeasy micro kit (*Qiagen*, Hilden, Germany) and homogenized. cDNA was purified and processed as previously described (27). Real-time PCR (RT-PCR) was performed for glucose transporters using a Rotor-Gene 6000 (*Qiagen*, Hilden, Germany). All primers for selected gene sequences were suggested by Primer-BLAST software [http://blast.ncbi.nlm.nih.gov/Blast.cgi](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.

**Barrier function and glucose transport assessment**

Briefly, an Ussing chamber was utilized with freshly isolated small intestine tissue using standard techniques (38). Intestinal tissues with an exposed surface area of 0.031 cm² were incubated in 5 ml of preheated 37°C Krebs buffer (140mM NaCl, 1.2mM MgCl₂, 1.2mM CaCl₂, 10mM KHCO₃, 0.2mM KH₂PO₄, and 1.2mM K₂HPO₄) on each side (serosal and mucosal), and pH was adjusted to 7.4. Each chamber was continuously oxygenated with O₂/CO₂ (95%/5%) and stirred by gas flow in the chambers. Transepithelial resistance (TER) was measured using previously described techniques (37). In addition, we examined a measure glucose transport as an indicator of absorptive function. Glucose transport is correlated to the uptake of sodium glucose transporter, thus leading to changes in ISC with glucose absorption. To assess glucose transport, 5ml of 10mM
D-glucose were added to the mucosal side, and 5ml of 10mM Mannitol to the serosal side. The injected short circuit current ($I_{sc}$) was monitored continuously as an indication of net active ion transport. Then the change of $I_{sc}$ was measured as following previous report (29).

**Blood sugar level, Serum Amylase and plasma GLP-2 level**

Since DPP4-I is a drug for type-2 diabetes mellitus, it was possible that glucose levels might decline with its usage. Therefore, we investigated blood glucose levels on post-operative days 3, 7, 14, 21, 30 and 90. We detected blood sugar levels using One touch Ultra (*Lifescan*, Milpitas, CA) 2 hours after drug administration. In addition, the use of DPP4-I has been shown to cause pancreatitis in some settings; thus, we also examined serum amylase levels at plasma GLP-2 at the end of the study. Blood was collected post-mortem. The blood samples for amylase were centrifuged 8000 rpm for 10 minutes in 4°C, and only serum was collected and tested. Plasma samples for GLP-2 were processed as previously described (23). Plasma GLP-2 was measured by radioimmunoassay using an antibody specific to the NH2 terminus of GLP-2. The results were expressed as pmol/L (13, 20).

**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% Na+Deoxycholate; 10 ug/ml BSA, 2 mM EDTA), containing 100 nM PMSF, 0.2 µl/ml aprotinin, and 5 µl/ml leupeptin. After homogenization and centrifugation at 14000g for 10 min at 4°C, the supernatant was stored in Læmmli loading dye. Equal amounts of protein content were separated on 4-20% SDS-polyacrylamide gels (Bio-Rad, Hercules, CA) and then transferred to a PVDF membrane. Blots were blocked in 5% bovine serum albumin and washed with Tris-buffered saline (TBS) containing 0.05% Tween (TBS-T) TBS-T. The membranes were incubated in each primary antibody (SGLT-1 [1:100], GLUT2 [1:100], [Santa Cruz Biotechnology, Santa Cruz, CA], GAPDH [1:1000] [Invitrogen; Grand Island, NY]) overnight at 4°C. After three washes with TBS-T, the membranes were incubated for 1 hour with secondary antibody. Following two additional washes with TBS-T, the membranes were developed for visualization of protein by the addition of enhanced chemiluminescence reagent using the manufacturer’s protocol (Supersignal® West Femto; *ThermoScientific*, Logan, UT).

**Statistics**

At least 5 animals were used per group per study as was determined by power analysis. Data are expressed as mean ± SEM. For comparison of two groups, unpaired T-tests were used, and ANOVA with a Bonferroni post-hoc test was used to determine the significance of differences when comparing more than one group. Each study group was compared to the Naïve group and to the placebo group of same time span. Differences between groups were tested using pairwise comparisons within the model. P values less than 0.05.
RESULTS

Determination of the optimal dose of DPP4-I in a mouse SBS model

Because this particular DPP4-I, MK-0626, had never been used for treatment of SBS, dosage optimization was performed over a short timeframe (3 days) of administration, a duration chosen because of our preliminary data with another DPP4-I (24). Merck (provider of MK-0626) suggested doses of 1 mg/kg and 3 mg/kg based on their previous data. We distributed mice into 5 groups: control (PBS only), 1 mg/kg once daily, 1 mg/kg twice daily (at 12 hours intervals), 3 mg/kg once daily, and 3 mg/kg twice daily given over 3 days. Body weight changes and blood glucose levels were not statistical significant between groups. All groups treated with DPP4-I had significantly increased IEC proliferation compared to placebo group in both jejunum and ileum except jejunum of 1mg/kg/day group (p<0.05) (TABLE 2). Body weight changes all declined, which is typical after a major intestinal resection, but none exceeded 18% from original weight.

We examined the morphology of the mucosa in all groups. The 1 mg/kg/daily group in jejunum along with the 1 mg/kg/daily group and 3 mg/kg/daily group in ileum exhibited statistically significant increases in crypt depth compared to the each placebo group (p<0.05) (TABLE 2). Villus height did not have any statistical significant change. Plasma GLP-2 levels were statistically increased (~2-fold) in all groups, and achieved significant elevation in the 1mg/kg/day and 3mg/kg/day group compared to placebo (p<0.05) (TABLE 2). We determined the efficiency of the glucose transport in jejunum and ileum as an intestine functional marker (TABLE 2). The 3mg/kg/twice daily group exhibited a significant increase in glucose transport in the jejunum and the ileum (p<0.05). Based on IEC proliferation and intestinal function, we selected 3mg/kg/twice daily as the optimal dose for the subsequent studies. For the remainder of the experiments we administered 3 mg/kg twice daily for 7 days, 30 days, and 90 days. Since we observed similar effects at 3 and 7 days that we also saw at 30 days, a an additional group of SBS mice were treated for 7 days followed by 23 days with removal of the DPP4-I. This is referred to as the DPP4-I 7 day/ PBS 23 day group.

DPP-IV-I stimulated epithelial cell proliferation in jejunum and ileum at all study time points

PCNA staining was performed to determine IEC proliferation at each time period. For all time periods, the SBS group showed increased IEC proliferation compared to naïve (non-operated upon) mice. DPP4-I significantly stimulated IEC proliferation beyond this adaptive state compared to most placebo groups in
jejunum and ileum (p<0.05) (FIGURE 2 and TABLE 3). While the dominant action of the drug was observed in the jejunum where a greater density of GLP-2 receptors are found, DPP4-I was also quite effective at promoting ileal IEC proliferation for all time points except at the 90 day period, where proliferation levels were similar between placebo and treatment groups.

DPP-IV-I treatment led to greater morphological adaptive changes

Intestinal morphology was examined in each group (FIGURE 3 and 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 for the 3, 7 and 30 day and DPP4-I 7 day/PBS 23 day groups for ileum. Administration of DPP4-I for 7 days provided the greatest effect in the jejunum. For villus height: DPP4-I 7 day 454.0±16.1 μm, placebo 7 day 404.1±13.2 μm, p<0.05; and for crypt depth: DPP4-I 7 day 135.8±5.2 μm, Placebo 7 day 106.1±2.8 μm, p<0.001). The DPP4-I 7 day/PBS 23 day group resulted in a 25% increase in villus height in jejunum, and although not significantly different from the other 7 day groups, it represented the largest increase of all groups. Although adaptation was somewhat less, the ileum had a statistically significant increase in crypt depth in the 7 day (p<0.05), 30 day (p<0.001) and DPP4-I 7 day / PBS 23 day (p<0.01) DPP-IV-I treatment groups.

DPP-IV-I promoted intestinal functional changes at an early phase 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 the addition of glucose to the mucosal side of the tissue. Interestingly, glucose transport changes were significantly up-regulated in both jejunum and ileum in the DPP4-I 3 day group compared to the placebo 3 day group (p<0.05) (FIGURE 4A). Isc changes were not significantly different for the other time periods studied.

As an additional approach to examining functional changes, a selected group of transporters which would reflect intestinal glucose transport were examined at the mRNA level of expression. In the ileum, mRNA abundance of SGLT1 and GLUT2 (FIGURE 4B and C) were significantly up-regulated in DPP4-I in the 3 day study group compared to placebo mice at 3 days (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 (FIGURE 4D).

We next confirmed the up-regulation of SGLT1 protein using western blotting techniques (FIGURE 5). SGLT1 expression was markedly increased in the DPP4-I 3-day group versus placebo for the same time period (p<0.05). However, levels of SGLT-1 were not significantly different from naïve controls. It is possible, that
the surgical resection of small bowel may normally down regulate this transporter (placebo group), but that this is up-regulated with the use of a DPP4-I. These results suggest that DPP4-I affects intestinal function at a very early phase after intestinal resection (3 days), and were not sustained in later time periods.

**DPP4-I drove an increase in GLP-2 plasma levels, and GLP-2R and IGF-1R mRNA expression.**

We next determined GLP-2R and IGF-1R mRNA expression by RT-PCR in each study group (TABLE 4) in order to examine for potential feedback response of blocking DPP4, and potential mechanisms which 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 group (p<0.05) and in the DPP4-I 7 day/PBS 23 day group (p<0.01) compared to 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 to the placebo 30 day group. As well, 3 day and 30 day values were not significantly different from controls. These results suggest that 7 days of an early drive toward absorption with this drug, and but continued proliferative effects beyond this time period.

Figure 6 shows serial plasma levels of GLP-2 as measured by radioimmunoassays. It was interesting to note that both placebo and DPP4-I treated groups showed an increase in GLP-2 levels compared to naïve controls; and this trend continued for the first 30 days. Although not completely significant, the DPP4-I groups showed higher GLP-2 levels over this time period; and this trended to significance by day 30. Interestingly, despite seeing improved adaptation in the 7 day DPP4-I/23 day placebo group, this increased GLP-2 was not seen in this group; potentially, the improved adaptation in the first 7 days had long-lasting effects without sustained GLP-2 levels (Figure 6). It was also interesting to note that both groups at 90 days showed a decline in GLP-2 levels, suggesting this group had entered a post-adaptive phase.

**Gross morphologic changes and Intestinal transepithelial resistance**

We next examined intestinal adaptation on a gross morphological basis and the effect of DPP4-I on epithelial barrier function (10). We calculated the intestinal diameter and circumference of transverse histologic sections. Diameters were not different from naïve mouse jejunum or ileum until 30 days. The DPP4-I 30 day group exhibited a significantly increased diameter and circumference compared to the placebo 30 day group (FIGURE 7A, B, and 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 DPP-IV-I failed to drive any further change compared to placebo.

Intestine from DPP4-I treated mice exhibited a statistically significant increase in TER at 30 days in the jejunum compared to placebo (p<0.05) (FIGURE 7D and 7E); whereas the DPP4-I 7 day/PBS 23 day group actually led to lower TER values. TER declined in the jejunal specimens from placebo mice at 90 days, and this was partially prevented with administration of DPP4-I. TER measurements in ileal specimens were
significantly decreased in most of the treatment groups compared to 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 in 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 groups were not significantly different between the two groups up to the 90 day time point. Interestingly, at 90 days, the DPP4-I group showed significant weight loss compared to the placebo group.

We examined the glucose level in blood taken from weekly tail vein samples. At post-operative day 14, glucose levels were markedly reduced in the DPP4-I group compared to the placebo group (Placebo: 190.0±13.1 mg/dl, DPP-IV-I 153.3±9.6, p<0.05); however, the glucose levels remained within a normal range. Glucose levels at other time periods were not statistically different (TABLE 5).

Long-term administration of DPP4-I did not lead to chemical evidence of pancreatitis

Because usage of DPP4-I has been linked to pancreatitis when used as a type-2 diabetes drug (36), we examined serum amylase levels. Serum amylase levels remained within normal range for all time periods (TABLE 5).

DISCUSSION

Short bowel syndrome (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 (31). Patient outcomes have been linked to the length and function of remaining small intestine (28); 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 (26) and produces various beneficial effects in an SBS model (25), indicating that long lasting increases in GLP-2 may be beneficial to healing SBS. However, long term GLP-2 treatment is very expensive, exceeding over 300,000 U.S. dollars per year and requires daily injection. To circumvent the cost of long-term GLP-2, we investigated a novel approach with a DPP-IV inhibitor. On average, the yearly cost of this class of drugs is well under $1,500 U.S. dollars, and thus a vast savings may be incurred with its use. DPP4 cleaves GLP-2 in 7 minutes in the serum; thus, blocking DPP-IV activity would increase endogenous GLP-2. We previously
examined the efficacy of short-term inhibition of the DPP4-I sitagliptin phosphate on repair of a mouse SBS model intestine (23). DPP4 inhibition enhanced the endogenous GLP-2 upon administration of a liquid diet in mice (14). Concordant with GLP-2 facilitating repair of the intestine, we found that 3 days of DPP4 inhibition increased plasma GLP-2 levels, cellular 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 felt 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 in animal studies (7), and may have similar adaptive action in our 50% proximal small bowel resection (SBR) mouse model than sitagliptin. To approach this, we used MK-0626 over 7, 30, or 90 days to access whether better repair efficacy 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. Here, 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 no proliferation data. However, it was interesting to note that crypt depth gain in human trials ranged from 17% to 64% over a 24 week period (18). Further, in a rat model of SBS, crypt cell numbers (depth not reported) increased by ~20 to 24% over the study period in the group treated with exogenously human GLP-2 (21). Thus exogenous GLP-2 was somewhat superior gains in our study of ~15% compared to placebo treated mice. This is not entirely surprising, but future studies will need to be done in a more head to head comparison of these two treatment options for SBS.

Swietlicki, et al. and Garrison, et al. have shown that exogenous GLP-2 and GLP-2 analogs facilitate intestinal adaptation at very early time points, with changes seen within 48 to 72 hours (11, 33). Concordant with these studies, we show here that DPP-IV inhibitors increased glucose uptake and morphological changes at 3 days of treatment. As well, our present study confirmed a 2-fold increase in circulating GLP-2 levels in mice receiving a DPP-IV-I, which is thus driving these levels beyond what the normal adaptive response. We also show that following 7 days of treatment then 23 days of no treatment the intestine continued to show signs of adaptation that were similar to that observed with just 7 days of treatment. The observed action of MK-0626 varied, in that this nutrient uptake was not observed after the first week, but actions on proliferation were continually observed even up to 90 days. Taken together, these data demonstrate that beneficial effects of DPP4 inhibition occur both in an early and late phase of adaptation, and may suggest a benefit from long-term usage. Further studies will need to be done to determine the most optimal dosing and duration of therapy if this strategy is taken to the clinical level.

To better understand the mechanisms of the DPP4-I/GLP-2 adaptive response, we studied the expression of GLP-2R, IGF-1, and the glucose transporter proteins SGLT1, GLUT5, and GLUT2. We detected an increase
of receptor expression for both GLP-2 and IGF-1 in our study with DPP-IV-I treatment, and this effect was predominantly noted in the jejunum. We also noted a significant increase in IGF-1 in the DPP-4-I treatment group at 7 days and the 7d/28d placebo groups in jejunal specimens. It was interesting that this part of the adaptive response was early, similar to the other adaptive changes noted. As has been previously appreciated, the dominant area for these receptors was in the jejunum (3). However, it was important to note that the adaptive action of DPP4-I was also observed in ileal specimens, similar to that seen by other investigators (22). Exogenous GLP-2 has also been shown by others to augment GLP-2R expression. In a report by Koopman, et al ileal GLP-2R expression rose to an even greater extent than jejunal (22). Here, we found that DPP4-I led to greater increases in expression of GLP-2R and IGF-1 in jejunal segments. 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 indicated that adaptation happens early and lasts for a considerable time after treatment has ended.

As the class of DPP4-I drugs are FDA approved as treatments for diabetes, and that 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 GIP. GLP-1 and GIP delay gastric emptying (16) which may reduce food intake (1), making DPP4-I weight neutral or leading to weight loss (36). In fact, in the post-operative 90 day period, body weights levels were lower in the DPP4-I group than the placebo group. However, glucose levels were not significantly different from placebo groups at all time periods. Despite this degree of safety, it will be important for patients to be closely monitored while receiving these inhibitors. As well, usage of DPP4-Is can stimulate pancreatic secretion, and thus, there will be a critical need for monitoring amylase and lipase levels. Again, amylase levels were consistently within or below the normal range for mice in the present study. One potential obstacle for the clinical usage of DPP4-I in SBS will be in those individuals who have lost a substantial portion of the distal ileum and right colon, the predominate source of L-cells which produce GLP-2 (8). 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 the action of those receiving exogenous GLP-2 derivatives.

In conclusion, our results indicate that use of DPP4-I for the treatment of SBS results in long-term adaptation benefits. The predominant benefit of optimal nutrient transport seems to occur early, but proliferative effect are still seen up to 3 months of therapy. The duration of the treatment may be somewhat long-lasting, as a relatively short DPP4-I treatment time of one week followed by treatment withdrawal, led to sustained efficacy. While restarting the treatment after this rest period may provide further adaptation, this will need to be the subject of future studies. It is hoped that these studies may prompt future clinical trials in the usage of these agents for patients suffering with SBS.
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Table 1. Primer design

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<td>GTGACATCCTCAGTTCTCTTTAG</td>
<td>GTGACATCCTCAGTTCTCTTTAG</td>
</tr>
<tr>
<td>GLUT5</td>
<td>CGTCTTTCCAGCCTCGGA</td>
<td>CGTCTTTCCAGCCTCGGA</td>
</tr>
<tr>
<td>GLUT5</td>
<td>TGCTGATCCAGAAGAAG</td>
<td>TGCTGATCCAGAAGAAG</td>
</tr>
<tr>
<td>GLP-2r</td>
<td>CACAGGGGAGGCTGAGGTG</td>
<td>CACAGGGGAGGCTGAGGTG</td>
</tr>
<tr>
<td>GLP-2r</td>
<td>GGAGGACCTGCGCTCGCA</td>
<td>GGAGGACCTGCGCTCGCA</td>
</tr>
<tr>
<td>IGF1</td>
<td>CGAATGTTCCCCCCAGCTGTT</td>
<td>CGAATGTTCCCCCCAGCTGTT</td>
</tr>
<tr>
<td>IGF1</td>
<td>GCCAGGGATAATGGAGCGA</td>
<td>GCCAGGGATAATGGAGCGA</td>
</tr>
<tr>
<td>IGF-1r</td>
<td>CGGAGCCTGCGGCGAGAACA</td>
<td>CGGAGCCTGCGGCGAGAACA</td>
</tr>
<tr>
<td>IGF-1r</td>
<td>ATCGCGATCCACACACCGGCC</td>
<td>ATCGCGATCCACACACCGGCC</td>
</tr>
<tr>
<td>β-Actin</td>
<td>TCTTTGCAGCTCCTCCTGTTGCA</td>
<td>TCTTTGCAGCTCCTCCTGTTGCA</td>
</tr>
<tr>
<td>β-Actin</td>
<td>TTGCACATGCGGAGCCGTTGTC</td>
<td>TTGCACATGCGGAGCCGTTGTC</td>
</tr>
</tbody>
</table>
### Table 2. Dose optimization of DPP-IV-I in SBS model

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo 6</td>
<td>6</td>
<td>83.5±1.2</td>
<td>122.3±13.2</td>
<td>45.7±4.9</td>
<td>48.3±4.4</td>
<td>403.3±11.8</td>
<td>282.4±20.5</td>
<td>117.4±4.7</td>
<td>103.0±3.7</td>
<td>7.0±1.3</td>
<td>9.3±4.8</td>
<td>-1.1±0.7</td>
</tr>
<tr>
<td>1mg/kg/day daily 6</td>
<td>6</td>
<td>88.7±1.2</td>
<td>145.3±10.8</td>
<td>53.4±5.3</td>
<td>58.8±2.1*</td>
<td>409.4±22.9</td>
<td>291.0±12.8</td>
<td>131.6±2.1 *</td>
<td>119.7±2.1 *</td>
<td>16.5±3.5 *</td>
<td>13.4±4.8</td>
<td>7.9±3.5</td>
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<tr>
<td>1mg/kg/day Q12h 6</td>
<td>6</td>
<td>85.6±0.8</td>
<td>153.2±8.2</td>
<td>60.0±1.9*</td>
<td>64.3±1.7*</td>
<td>411.2±8.4</td>
<td>278.8±10.1</td>
<td>129.2±4.3</td>
<td>115.1±6.1</td>
<td>14.0±3.1</td>
<td>12.1±11.7</td>
<td>4.8±4.1</td>
</tr>
<tr>
<td>3mg/kg/day daily 6</td>
<td>6</td>
<td>85.2±1.5</td>
<td>128.2±9.3</td>
<td>64.0±1.3*</td>
<td>55.8±3.5</td>
<td>373.4±23.0</td>
<td>296.1±7.1</td>
<td>123.4±7.6</td>
<td>117.7±4.1 *</td>
<td>15.7±2.7 *</td>
<td>25.4±7.4</td>
<td>-1.5±2.1</td>
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<tr>
<td>3mg/kg/day Q12h 6</td>
<td>6</td>
<td>84.2±1.0</td>
<td>127.8±16.5</td>
<td>60.2±3.5*</td>
<td>62.1±4.4*</td>
<td>410.8±10.8</td>
<td>298.2±12.3</td>
<td>119.4±3.8</td>
<td>114.1±5.1</td>
<td>12.2±3.2</td>
<td>64.3±22.0 *</td>
<td>37.0±12.3 *</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. Q 12h denotes dosing every 12 hours.

* p<0.05 compared to placebo group. **Results are the percent of the baseline (preoperative) weight.
Table 3. The morphological and cell proliferation changes

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Villus height</th>
<th>Crypt depth</th>
<th>PCNA Positive rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve</td>
<td>6</td>
<td>305.7±11.4</td>
<td>99.7±2.5</td>
<td>35.9±1.1</td>
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<tr>
<td>Placebo 3day</td>
<td>6</td>
<td>282.4±20.5</td>
<td>103.0±3.7</td>
<td>45.4±2.7 *</td>
</tr>
<tr>
<td>DPP4-I 3day</td>
<td>6</td>
<td>298.2±12.3</td>
<td>114.1±5.1</td>
<td>58.1±2.1 *†</td>
</tr>
<tr>
<td>Placebo 7day</td>
<td>6</td>
<td>263.8±16.3</td>
<td>101.2±4.1</td>
<td>38.9±3.3</td>
</tr>
<tr>
<td>DPP4-I 7day</td>
<td>6</td>
<td>290.9±13.3</td>
<td>115.1±4.5</td>
<td>54.6±1.6 *†</td>
</tr>
<tr>
<td>Placebo 30day</td>
<td>5</td>
<td>256.8±22.6</td>
<td>93.1±2.1</td>
<td>43.4±1.1 *</td>
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<tr>
<td>DPP4-I 30day</td>
<td>7</td>
<td>275.8±5.2</td>
<td>108.8±1.2</td>
<td>53.5±1.3 *†</td>
</tr>
<tr>
<td>DPP4-I 7day / PBS23day</td>
<td>6</td>
<td>307.1±39.5</td>
<td>110.2±4.7</td>
<td>52.4±4.4 *</td>
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<tr>
<td>Placebo 90day</td>
<td>6</td>
<td>344.1±14.3</td>
<td>104.6±6.3</td>
<td>49.1±1.2 *</td>
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<tr>
<td>DPP4-I 90day</td>
<td>5</td>
<td>322.2±20.0</td>
<td>119.3±5.0</td>
<td>48.6±1.3 *</td>
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</tbody>
</table>

Results are expressed as mean ± SEM of ileal samples.
* p<0.05 for comparing with Naïve group
† p<0.05 for comparing with Placebo group
### Table 4. The effects of DPP4-I on GLP-2R and IGF-1

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>GLP-2r mRNA expression in Jejunum</th>
<th>GLP-2r mRNA expression in Ileum</th>
<th>IGF-1 mRNA expression in Jejunum</th>
<th>IGF-1 mRNA expression in Ileum</th>
<th>IGF-1r mRNA expression in Jejunum</th>
<th>IGF-1r mRNA expression in Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naïve group</td>
<td>6</td>
<td>6.9±2.6</td>
<td>4.6±0.5</td>
<td>8.5±3.0</td>
<td>10.0±5.0</td>
<td>10.5±1.5</td>
<td>16.8±10.2</td>
</tr>
<tr>
<td>Placebo 3day</td>
<td>6</td>
<td>15.4±5.4</td>
<td>7.4±3.2</td>
<td>25.7±18.4</td>
<td>8.0±3.0</td>
<td>18.0±5.6</td>
<td>12.4±3.0</td>
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<tr>
<td>DPP4-I 3day</td>
<td>6</td>
<td>7.1±1.9</td>
<td>11.5±4.0</td>
<td>37.5±19.2</td>
<td>24.9±12.1</td>
<td>11.5±2.4</td>
<td>26.7±13.7</td>
</tr>
<tr>
<td>Placebo 7day</td>
<td>6</td>
<td>12.0±3.1</td>
<td>4.0±1.2</td>
<td>18.0±11.4</td>
<td>16.4±7.8</td>
<td>17.6±4.2</td>
<td>9.2±1.9</td>
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<tr>
<td>DPP4-I 7day</td>
<td>6</td>
<td>30.1±5.9*#</td>
<td>5.5±2.0</td>
<td>64.1±21.4*</td>
<td>11.1±3.9</td>
<td>33.7±9.3*</td>
<td>12.5±2.9</td>
</tr>
<tr>
<td>Placebo 30day</td>
<td>5</td>
<td>7.5±1.6</td>
<td>5.4±1.5</td>
<td>9.0±1.8</td>
<td>6.0±1.8</td>
<td>8.5±1.1</td>
<td>13.8±1.2</td>
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<tr>
<td>DPP4-I 30day</td>
<td>7</td>
<td>15.8±6.1</td>
<td>4.2±1.3</td>
<td>11.4±5.0</td>
<td>5.2±1.1</td>
<td>14.7±3.8</td>
<td>10.0±1.9</td>
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<tr>
<td>DPP4-I 7day/PBS</td>
<td>6</td>
<td>72.9±15.8*#</td>
<td>20.0±7.8</td>
<td>118.1±34.4*#</td>
<td>19.6±6.3</td>
<td>84.5±11.5*#</td>
<td>14.3±0.8</td>
</tr>
</tbody>
</table>

* p<0.05 Compared to placebo group. Each data are divided 10^3. Results are expressed as mean ± SEM. Fold changes of target genes were calculated using compared quantification to β-actin results. *p<0.05 compared with the Naïve group #p<0.05 compared with the Placebo group.
<table>
<thead>
<tr>
<th>Post-operative day</th>
<th>Placebo group</th>
<th>DPP4-I group</th>
<th>p-Value</th>
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</thead>
<tbody>
<tr>
<td>Body weight [mean grams]</td>
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<tr>
<td>3days</td>
<td>23.1±0.4</td>
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<td>7days</td>
<td>20.1±0.7</td>
<td>20.5±0.9</td>
<td>0.75</td>
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<td>14days</td>
<td>19.6±0.8</td>
<td>21.3±0.4</td>
<td>0.07</td>
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<td>21days</td>
<td>22.6±0.8</td>
<td>21.0±1.1</td>
<td>0.29</td>
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<tr>
<td>30days</td>
<td>22.9±1.0</td>
<td>22.3±1.3</td>
<td>0.75</td>
</tr>
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<td>90days</td>
<td>23.2±0.9</td>
<td>22.6±1.2</td>
<td>0.71</td>
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<tr>
<td>Blood glucose level [mg/dl]</td>
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<td></td>
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<tr>
<td>3days</td>
<td>122.3±13.2</td>
<td>127.8±16.5</td>
<td>0.8</td>
</tr>
<tr>
<td>7days</td>
<td>139.3±12.8</td>
<td>149.3±19.4</td>
<td>0.34</td>
</tr>
<tr>
<td>14days</td>
<td>190.0±13.1</td>
<td>153.3±9.6</td>
<td>&lt;0.05</td>
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<td>21days</td>
<td>187.2±13.1</td>
<td>175.9±14.0</td>
<td>0.58</td>
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<td>30days</td>
<td>169.2±15.9</td>
<td>181.9±12.0</td>
<td>0.53</td>
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<tr>
<td>90days</td>
<td>181.8±16.4</td>
<td>162±26.9</td>
<td>0.54</td>
</tr>
<tr>
<td>Serum Amylase level [U/L]</td>
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<tr>
<td>3days</td>
<td>-</td>
<td>1311±79.5</td>
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<td>7days</td>
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<td>30days</td>
<td>-</td>
<td>1504±217</td>
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</tr>
<tr>
<td>90days</td>
<td>-</td>
<td>1445±171.8</td>
<td></td>
</tr>
</tbody>
</table>

*Results are expressed as mean ± SEM.
*p-value was compared to each corresponding placebo group.
*Normal blood glucose levels range from 60-130 mg/dl
*Normal serum amylase levels range from 2000-6000 U/L
Figure Legends

FIGURE 1. Surgical and tissue harvest schematic.

A 50% proximal small bowel resection was performed. This allowed for preservation of potential GLP-2 sources in the distal small bowel. The harvesting scheme for tissue acquisition is also shown. Basically two sections were taken for histological and TER analysis. The remainder was used for RT-PCR and protein.

FIGURE 2. Intestinal epithelial cell proliferation in jejunum and ileum.

Intestinal epithelial cell (IEC) proliferation was measured by immunofluorescence staining for PCNA, and is expressed at the percent of PCNA positive crypt cells per total number of crypts. DPP4-I significantly led to an increase in IEC proliferation vs. placebo groups in all time periods in jejunum and ileum. The placebo 30 day in jejunum, the placebo 3 day and 30 day group in ileum also markedly exhibited increase of cell proliferation compared to Naïve group. All time points were obtained using 3 mg/kg/ every 12 hour dosing of DPP4-I. N=5 or 6 per group. Statistical analysis by ANOVA, *p<0.05 compared to naïve; †p<0.05 compared to corresponding placebo.

FIGURE 3. Small bowel histomorphological adaptation.

Histomorphologic adaptation of the jejunum is shown (see Table 3 for ileum) as mean±SEM for villus height and crypt depth. Note a significant effect of DPP4-I is seen on villus height at the 7 and 30 day study period, but a significant increase in adaptive effect is seen by an increase in crypt depth at every study time point. All time points were obtained using 3 mg/kg/ every 12 hour dosing of DPP4-I. N=5 or 6 per group. Statistical analysis by ANOVA, *p<0.05 compared to naïve; †p<0.05 compared to corresponding placebo.

FIGURE 4. DPP-IV-I promoted the intestinal function at an early phase.

A: Changes in isoelectric current (Isc) was determined in Ussing chambers by the mucosal application of glucose, and was used as a functional measure of glucose transport. Isc changes were statistically up-regulated in jejunum and ileum in the DPP-IV-I 3 day group. #p<0.05 compared to corresponding placebo.

B, C and D: Abundances of nutrient transporters were measured at the RNA level (SGLT-1, GLUT2 and GLUT5) using RT-PCR. SGLT-1 (B) and GLUT2 mRNA expression (C) were significantly up-regulated in DPP4-I 3 day study group compared to the Placebo 3 day group. There was no effect on GLUT5 expression noted. These results may indicate that DPP4-I affect intestinal function in early phase. Statistical analysis by ANOVA, *p<0.05 compared to corresponding placebo. N=6 specimens per group.

FIGURE 5. DPP-IV-I associated changes in SGLT-1.

The nutrient transporter SGLT-1 was measured using western blotting. The SGLT-1 expression exhibited
markedly increase in the DPP4-I 3day group compared to placebo controls (p<0.05). SGLT-1 expression in the placebo group was significantly decreased compared to the Naïve (non-operated) group. Statistical analysis by ANOVA, N=6 specimens per group. Note all of the gel images were run on the same gel and at the same time; however, the two representative gels for the Naïve group are shown separately to remove a single column which yielded a poor image and blot.

**FIGURE 6.** Plasma GLP-2 levels were determined at the time of harvesting from each study time period. Note GLP-2 levels progressively rose after SBS creation, but were significantly higher in the DDP4-I group compared to the placebo group, and this effect was greatest at the 30 day treatment period. *P<0.05; #P=0.06.

**FIGURE 7.** Gross morphologic and transepithelial barrier function changes.

**A, B and C:** Intestinal diameter and circumference were measured at low magnification using H&E stained slides. The DPP4-I 30day group and DPP4-I 7day / PBS 23day group exhibited significant increased diameter and circumference compare to the placebo 30day. *p<0.05 compared to naïve; †p<0.05 compared to 30 day placebo.

**D:** Jejunal transepithelial resistance (TER in Ω • cm2) was measured in Ussing chambers from full-thickness small bowel segments. DPP4-I led to a statistically significant increase in the 30day jejunum segments compared to 30day placebo specimens (p<0.05). †p<0.01 compared to 30 day DPP4-I; ††p<0.05 compared to 30 day DPP4-I.

**E:** Ileal TER results. Note, the ileum TER in the DPP4-I 30day group was significantly decreased compared to the placebo 30day group (*p<0.05 compared to naïve group).
REFERENCES


PCNA / DAPI

Fig 2

Epithelial cell proliferation - Jejunum

Epithelial cell proliferation - Ileum
Fig 3
A. Glucose transport change in jejunum

B. SGLT1 in Jejunum

C. GLUT2 in Jejunum

D. GLUT5 in Jejunum

figure4
SGLT1 / β-Actin protein expression

figure5
Plasma GLP-2 (PM)

- Naive
- 3 Day placebo
- 5 Day DPP4-1
- 30 Day placebo
- 7d DPP4-1
- 30 Day DPP4-1
- 23d placebo
- 90 Day placebo
- 90 Day DPP4-1