Journal of Parenteral and Enteral Nutrition
Volume 43 Number 7
September 2019 891–898

DOI: 10.1002/jpen.1500
wileyonlinelibrary.com
WILEY

Novel Long-Acting GLP-2 Analogue, FE 203799 (Apraglutide), Enhances Adaptation and Linear Intestinal Growth in a Neonatal Piglet Model of Short Bowel Syndrome with Total Resection of the Ileum

George M. Slim, MB BCh[1]; Marihan Lansing, MB BCh[1]; Pamela Wizzard, BSc RAHT[1]; Patrick N. Nation, DVM[2]; Sarah E. Wheeler, MSc[3]; Patricia L. Brubaker, PhD, FRSC[3]; Palle B. Jeppesen, MD, PhD[4]; Paul W. Wales, MD, MSc[1,5,6]; and Justine M. Turner, MBBS, PhD[1]

Abstract

Background: Glucagon-like peptide-2 (GLP-2) is an intestinotrophic factor released from L-cells in the ileum, a segment commonly resected or atretic in neonatal short bowel syndrome (SBS). In piglets, ileal resection decreases intestinal adaptation and endogenous GLP-2 production, whereas exogenous replacement promotes adaptation. In this study, we determined the effect of a novel long-acting GLP-2 analogue, FE 203799 (FE; apraglutide), upon intestinal growth, adaptation, and function in neonatal SBS piglets without ileum. *Methods:* Neonatal piglets were randomized to saline (n = 10) vs FE treatment (n = 8). All piglets underwent 75% intestinal resection with jejunocolic anastomosis and were pair-fed parenteral and enteral nutrition. Saline and FE (5 mg/kg) treatments were administered subcutaneously on days 0 and 4. On day 6, 24-hour fecal samples were collected for subsequent nutrient analysis. On day 7, small-intestinal length and weight were measured and tissue collected for analyses. *Results:* On day 7, saline and FE-treated piglets were healthy and gained equivalent weight ($P = 0.12$). Compared with saline piglets, FE-treated piglets had lower fecal fat ($P = 0.043$) and energy ($P = 0.043$) losses and exhibited intestinal lengthening ($P = 0.001$), greater small-intestinal weight ($P = 0.004$), longer villus height ($P = 0.027$), and greater crypt depth ($P = 0.054$). *Conclusions:* The subcutaneous GLP-2 analogue, FE, enhanced intestinal adaptation in a neonatal model of SBS without ileum. The observed intestinal lengthening with FE treatment was unique compared with our prior experience with native GLP-2 in this same model and has important clinical implications for treating neonatal SBS. At this developmental stage, growth in the intestine, if augmented, could accelerate weaning from parenteral nutrition. (*JPEN J Parenter Enteral Nutr.* 2019;43:891–898)

Clinical Relevancy Statement

Children with short bowel syndrome are dependent on parenteral nutrition (PN) for growth and development and are at risk of the associated complications such as sepsis and liver disease. To achieve enteral autonomy, the remnant intestine must adapt both structurally and functionally. FE 203799 (FE; apraglutide) treatment has the clinical

From the [1]Department of Pediatrics, University of Alberta, Edmonton, Alberta, Canada; [2]Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada; [3]Departments of Physiology and Medicine, University of Toronto, Toronto, Ontario, Canada; [4]Department of Medical Gastroenterology, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark; [5]Division of General Surgery, Hospital for Sick Children, Toronto, Ontario, Canada; and the [6]Group for Improvement of Intestinal Function and Treatment, Hospital for Sick Children, Toronto, Ontario, Canada.

Financial disclosure: P. L. Brubaker is supported by the Canada Research Chairs Program. Some of the equipment used in this study was supported by the Diet, Digestive Tract, and Disease Centre funded by the Canadian Foundation for Innovation and Ontario Research Fund (project #19442 and #3096). Research was funded by Glypharma Therapeutic Inc./Therachon. Glypharma Therapeutic Inc. provided FE 203799 in kind and funded the experimental work. All experiments were designed by J. M. Turner and P. Wizzard, without direction from the industry partner. All experimental work, data analysis, and preparation of the manuscript were undertaken independently of the industry partner.

Conflicts of interest: None declared.

Received for publication October 25, 2018; accepted for publication December 10, 2018.

This article originally appeared online on January 6, 2019.

Corresponding Author:
Justine Turner, MBBS, PhD, Department of Pediatrics, University of Alberta, 4–595, Edmonton Clinic Health Academy, Edmonton, AB, T6G 1C9, Canada.
Email: justine.turner@albertahealthservices.ca

advantage of ease of administration, with the potential for less frequent injections than currently available glucagon-like peptide-2 (GLP-2) analogues. In this neonatal model, FE enhanced intestinal adaptation, reduced nutrient losses, and promoted gut lengthening. Neonates have a unique potential for intestinal growth in length, which may be a critical factor for sustaining autonomy from PN, particularly once trophic therapies are discontinued. In this study, the effect on growth in length was unexpected compared with our prior experience with this animal model using an intravenous native GLP-2 analogue. This warrants further investigation and comparison with other clinically available GLP-2 analogues.

Introduction

Massive resection of the intestine results in short bowel syndrome (SBS), characterized by a reduced and functionally insufficient or failing intestinal remnant for survival, growth, and hydration.[1] Although long-term parenteral nutrition (PN) is currently essential for survival and growth in neonates with SBS and intestinal failure, it is also associated with prolonged hospitalization and recurring central venous catheter complications, sepsis, and cholestatic liver disease. The extensive morbidity and mortality accompanying SBS, the significant health-related costs, and the poor quality of life for children and their families merit the need to wean patients off PN and assist them in attaining enteral independence as soon as possible.[2]

The residual anatomy in neonates with SBS is a key predictor of time needed to wean them off PN and achieve enteral autonomy.[3,4] With this in mind, we developed neonatal piglet models of SBS that have both total ileal resection (with jejunocolic [JC] anastomosis) and mid-intestinal resection leaving equal jejunum and ileum (jejunoileal [JI] anastomosis).[5] Using these models, we have consistently shown that the JC model, when compared with the JI model, is associated with poor adaptation and longer time required to wean patients off PN. A large cohort of 272 infants also showed that removal of the highly adaptive ileum and ileocecal valve occurs in 52% of cases, and given this anatomy, infants were less likely to achieve enteral autonomy.[3]

Using our neonatal piglet models, we have shown that ileal resection (JC anatomy) is also associated with reduced secretion of glucagon-like peptide-2 (GLP-2).[6] GLP-2 is a 33-amino-acid peptide that, upon nutrient ingestion, is secreted by enteroendocrine L-cells of the distal ileum and colon.[7] In piglets[8] and in humans[9,10] with intestinal resection, exogenous GLP-2 treatment has been shown to increase intestinal adaptation, reduce fecal output, and allow weaning of PN.[11,12] Recently, a GLP-2 analogue, teduglutide, has been approved for use in adults with SBS. Preliminary trials with promising results have also been conducted in children, and pediatric approval (≥1 year old) has been granted in the European Union.[9] In children with SBS, teduglutide treatment allowed a median reduction of 41% and 45% in prescribed PN volume and energy, respectively.[9]

Currently, there is no Food and Drug Administration–approved treatment that enhances both gut lengthening and adaptation in the pediatric SBS population. Although GLP-2 and teduglutide are both being evaluated for pediatric use, there appears to be a limited effect on gut lengthening, based on animal data.[7,11,13] The greatest potential for lengthening, both with and without GLP-2 treatment, appears to be seen in preterm piglets and in term piglets receiving higher enteral nutrition delivery.[11,13] Neonates have a unique potential for gut lengthening, which should be a focused target of any trophic therapy to increase the likelihood of weaning from PN. Although we know that the trophic effects on the mucosa may require sustained treatment, in both animals and humans treated with GLP-2.[14,15] Consistent with postnatal intestinal growth, it is plausible that neonatal gut lengthening would have the potential to persist when trophic therapies are discontinued, although this remains to be experimentally proven.[11,16,17] This issue is critical for the neonate with SBS, given the potential lifetime exposure to trophic therapies, with the potential for cancer or other risks.[18] We aimed to assess the adaptive potential, especially for gut lengthening, of a novel GLP-2 analogue—FE 203799 (FE), or apraglutide—in our JC piglet model of SBS. Apraglutide is a synthetic 33-amino-acid peptide with a molecular structure designed to preserve optimal pharmacological activity while increasing the half-life as compared with the native GLP-2 or other GLP-2 analogues. Hence, the half-life following subcutaneous administration in humans and various animal species is approximately 30 hours, allowing the possibility for only once-to-twice weekly treatment.

Methods

All procedures in this study were approved by the University of Alberta Animal Care and Use Committee for Livestock.

Animals and Surgical Procedures

Newborn Duroc piglets, 2–5 days old and weighing between 2–2.6 kg, were obtained from the Swine Research & Technology Centre and allocated to the following experimental groups: saline-treated (n = 10) compared with FE-treated (5 mg/kg/dose; n = 8). All saline and drug treatments were administered twice, subcutaneously, on days 0 and 4 postsurgery. This dose was chosen based on prior pharmacokinetic studies in adult mini pigs (data from Glypharma).

Under general anesthesia, piglets had intestinal surgery to measure the small-intestine length (using a silk suture, along the antimesenteric border, with minimal traction),

and all underwent a 75% distal intestinal resection with JC anastomosis (removing the entire ileum and a small portion of the right colon); a gastrostomy tube was inserted for enteral nutrition and a left central jugular venous catheter for PN.[5]

Piglets were then maintained in the laboratory as previously reported and housed in metabolic cages in heat-controlled and light-controlled rooms.[5] Our standard protocol includes pain relief, single-dose administration of the antibiotics florfenicol (Nuflor) and ampicillin at surgery to prevent catheter sepsis, heparin for catheter patency, and standardized health monitoring. We provided additional doses of antibiotics (ampicillin) to any piglet on trial that showed signs of sepsis, such as fever, lethargy, or vomiting. Weight measurements and fluid-balance calculations were conducted daily.

The isocaloric and isonitrogenous PN commenced immediately postoperatively, using a solution formulated for neonatal piglets (provides 15 g/kg/d amino acids, 10 g/kg/d lipid, 29 g/kg/d glucose). The metabolic requirements of a neonatal piglet are 5 times that of a human neonate; therefore, the delivery of 10 g/kg/d of parenteral lipid is equivalent to 2 g/kg/d in an infant. Continuous PN was administered by pressure-sensitive volumetric pumps. On day 2 postoperatively, gastric enteral nutrition commenced and was, similarly, continuously delivered. It is identical to PN, except polycose is substituted for glucose to reduce osmolality. The rate was 20% of target requirements, which is optimal to enhance the benefit of trophic therapy based on our data.[5] PN was continued at 100% of total requirements, given our prior experience with JC piglets being at risk of diarrhea with possible dehydration without continued PN support.

Piglets underwent terminal laparotomy at the end of the trial (day 7), when small-intestinal length was measured and compared with the postresection measurement from the initial surgery. In addition, the emptied small bowel was weighed, and jejunal tissue and scrapings (measured on a standardized length board for accurate mucosal weight, per cm) were collected.

Assessment of Fecal Nutrient Losses

On study day 6, fecal effluent of piglets was collected into drainable ostomy appliances for 24 hours (Hollister, Aurora, Ontario, Canada). A qualitative assessment was made of stool consistency: 0 indicated formed stool, 1 indicated semiformed stool, 2 indicated low volume watery stool, and 3 indicated large volume watery stool. Nitrogen,[19] carbohydrate,[20] and fat[21] contents were determined on the fecal effluent. A sample was freeze-dried for measurement of energy content by bomb calorimetry (model C 4000 A; IKA-Analysentechnik, Heitersheim, Germany) to determine fecal energy.[22]

Assessment of Structural Adaptation

Sections of the small intestine (jejunum) were obtained for histology 20 cm distal to the ligament of Treitz. Paraffin-mounted 5-μm sections from each intestinal site were stained with hematoxylin and eosin prior to assessment using a micrometer eyepiece (Nikon Eclipse 80i; Nikon, Tokyo, Japan). Mucosal hyperplasia was assessed by measurement of villus height and crypt depth, performed by a board-certified animal pathologist (P. N. Nation) blinded to treatment group. Height was taken from villi in longitudinal section; crypt depth was taken from the same area. Ten measurements per villus-crypt axis were used to calculate the mean (SD).

Molecular Analysis

Total RNA was isolated from a 2-mm section of freshly thawed jejunal mucosa collected by scraping (RNeasy Plus Mini Kit with Qiashredder; Qiagen Inc., Toronto, Canada) or using whole thickness jejunal sections. Total RNA was reverse transcribed using the 5X All-In-One Reverse Transcriptase Mastermix (Applied Biological Materials Inc., Richmond, Canada), and quantitative real-time polymerase chain reaction was conducted using Taqman Fast Mix Gene Expression Assays with the primers (Life Technologies, Carlsbad, CA) shown in Table S1. Primers noted with an asterisk were synthesized using the Custom TaqMan Assay Design Tool from Thermo Fisher Scientific (Waltham, MA). As previously validated, 18S rRNA was used as the internal control, and analysis was conducted using the $\Delta\Delta$Ct method.[23,24]

Statistical Analysis

Because not all data were normally distributed, the data are expressed as median and interquartile ranges (25th–75th), and comparisons were made using Mann-Whitney U tests; significance was set at P-value <0.05. All statistical analyses were performed using SPSS for Windows (version 24; SPSS Inc, and IBM Company, Chicago, IL, USA).

Results

Clinical Outcomes

At baseline, piglets were not different according to age (saline: 3 d [3.0–4.0]; FE: 3.5 d [2.2–4.0]; $P = 0.88$), weight (saline: 2.2 kg [2.1–2.4]; FE: 2.3 kg [2.1–2.4]; $P = 0.41$), or small-bowel length (saline: 557 cm [503–583]; FE: 554 cm [533–578]; $P = 0.92$). Over the study period, saline and treated groups gained similar weight (saline: 1.3 kg [1.1–1.4]; FE: 1.5 kg [1.2–1.6]; $P = 0.12$). During the trial, 8 piglets had clinical suspicion of sepsis, and 5 grew an organism on blood culture, which was taken as confirmation of line sepsis. This included 1 saline piglet (positive blood

Table 1. Nutrient Absorption.

Fecal Nutrient Data	Saline n = 9	FE-Treated, 5 mg/kg n = 8	*P*-Value
Fecal dried weight, g/24 h	3.24 (1.99–6.50)	5.96 (1.80–7.22)	0.76
% Dried fecal weight	9.10 (7.01–10.64)	7.15 (5.36–8.20)	0.21
Measured total energy, J/g	2187 (1928–2710)	1697 (1297–1976)	0.07
Calculated total energy, J/g	1992 (1574–2162)	1328 (1058–1514)	0.04
Fat, mg/g	29.2 (26.4–33.6)	20.1 (15.2–24.6)	0.04
Carbohydrate, mg/g	6.6 (4.6–35.4)	8.8 (4.0–10.9)	0.50
Nitrogen, mg/g	2.1 (1.7–3.2)	2.2 (1.7–3.0)	1.0

Data are represented as median and (interquartile range) and analyzed using Mann-Whitney U test.
FE, FE 203799.

culture for *Enterococcus faecalis)* that could not be treated successfully and so underwent humane euthanasia. Hence, all final results include only 9 saline piglets. All remaining piglets with suspicion of sepsis were successfully treated. This included 4 piglets in the FE group, 2 with positive blood cultures (*E. faecalis* and *Candida lusitaniae*), and 3 other saline control piglets, 2 with positive blood cultures (*E. faecalis* and *C. lusitaniae*).

Fecal Nutrient Losses

On day 5, 75% of FE-treated piglets (n = 6) had low-volume watery stool (score of 2) and the remainder high-volume watery stool (score of 3). This was similar to the saline piglets, with 67% (n = 6) having low-volume watery stool and the remainder high-volume watery stool. Results from fecal analysis are shown in Table 1. Overall stool dried weight (saline: 3.24 g [1.99–6.50]; FE: 5.96 g [1.80–7.22]; $P = 0.76$) and % dry weight (saline: 8.09% [6.71–11.09]; FE: 6.34% [4.71–8.76]; $P = 0.75$) were not different between groups.

Calculated fecal energy is based on the sum of measured individual nutrient losses from fat, carbohydrate, and protein. In Table 1, calculated fecal energy can be seen to be similar to the measured fecal energy, although it is consistently lower. The saline group compared with the FE-treated group had increased calculated fecal energy (saline: 1992 J/g [1574–2162]; FE: 1328 J/g [1058–15,146]; $P = 0.043$) and fecal fat (saline: 29.2 mg/g [26.4–33.6]; FE, 20.1 mg/g [15.2–24.6]; $P = 0.043$). The remaining fecal nutrient data did not statistically differ between the groups. The percent median difference in measured and calculated fecal energy between saline-treated and FE-treated piglets was on average 29% and 50%, respectively. Similarly, median fecal fat was on average 45% for saline compared with FE-treated piglets.

Structural Adaptation

Absolute growth in small-intestinal length was consistently greater in FE-treated piglets as compared with saline-treated piglets (saline: −4.5 cm [−10.2 to −1.5 cm]; FE: 11.5 cm [9.0–18.0]; $P = 0.001$). Indeed, none of the saline-treated piglets had growth in intestinal length, accounting for plausible measurement variability, the impact of adhesions, or the loss of small-bowel tonicity by day 7; ie, the lengths consistently measured less than compared with that following the initial resection surgery. Absolute small-bowel weight (saline: 23.6 g [22.6–24.8]; FE: 28.6 g [25.6–33.4]; $P = 0.005$), length (saline: 134.2 cm [128.9–141.4]; FE: 149.5 cm [143.8–161.9]; $P = 0.003$), and mucosal mass (saline: 113.3 mg/cm [108.5–141.8]; FE: 170.2 mg/cm [143.7–216.5]; $P = 0.012$) were all greater in the FE-treated group. Jejunum villus height (saline: 0.68 mm [0.56–0.79]; FE: 0.87 mm [0.71–0.94]; $P = 0.027$) and crypt depth were also increased with treatment (saline: 0.14 mm [0.13–0.15]; FE: 0.15 mm [0.14–0.17]; $P = .054$). Data are presented in Table 2. Figure 1 shows representative piglet histology from both groups.

Molecular Analysis

The results of the analysis of intestinal mucosal mRNA are shown in Figure 2. No differences were observed for any of the transcripts for tight junction proteins (*cldn 2*, *cldn 7*, *cldn 15*), nutrient absorption (*Fatp 4*, *Cd36*, *Slc5a1*, *Slc2a2*), or enteroendocrine proteins (*Gcg*, *Igf-1*, *Egf*) or their receptors (*Glp-2r*, *Igf-1r*, *Egfr*). A trend may be observed for *Fatp 4* expression to be increased in the mucosa of FE-treated piglets (saline: 1.02 [0.87–1.13]; FE: 1.22 [1.08–1.59]; $P = 0.08$). Similarly, no differences

Table 2. Structural Adaptation.

Morphometric Data of the Small Intestine	Saline n = 9	FE-Treated , 5 mg/kg n = 8	*P*-Value
Pre-resection length, cm	557 (503–583)	554 (533–578)	0.92
Postresection length, cm	140 (133–146)	138 (133–144)	0.63
Length posttreatment, cm	133 (127–141)	150 (144–162)	0.001
Absolute change in length, cm	−4.5 (−10–1.5)	12 (9–18)	0.001
Small bowel weight, g	23 (22–25)	29 (26–33)	0.01
Mucosal mass, g/cm	0.12 (0.10–0.14)	0.15 (0.12–0.19)	0.04
Villus height, mm	0.69 (0.56–0.76)	0.88 (0.71–0.94)	0.03
Crypt depth, mm	0.14 (0.14–0.15)	0.15 (0.14–0.17)	0.05

Data are represented as median and (interquartile range) and analyzed using Mann-Whitney U test.
FE, FE 203799.

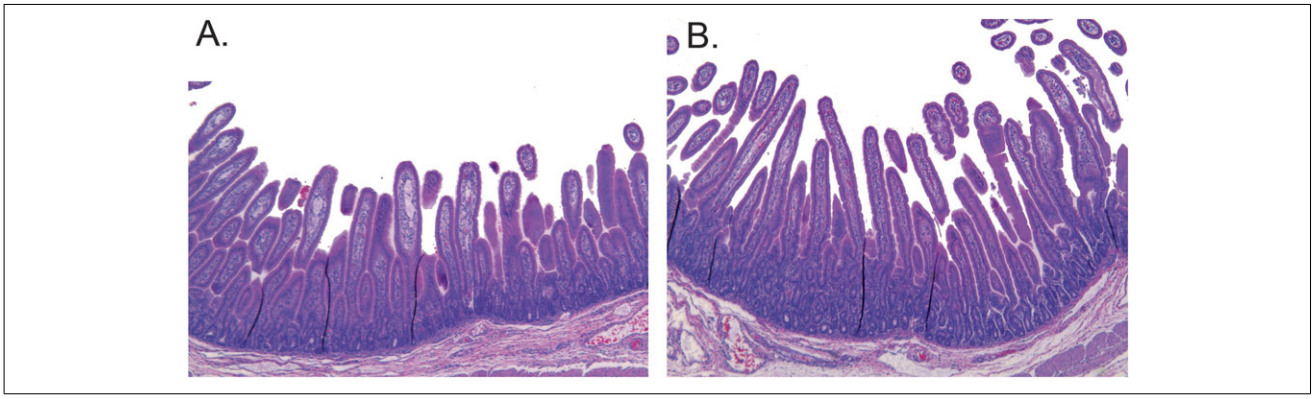

Figure 1. Representative samples of jejunum from JC piglets (magnification = 20×). (A) Normal jejunum in control-treated piglet. (B) Jejunum in FE 203799 (FE)–treated piglet with elongated villi compared with control.

were detected when whole intestinal sections, including the muscle layers, were analyzed (data not shown).

Discussion

To our knowledge, this study is the first to report the effects of the novel subcutaneous GLP-2 analogue, FE (apraglutide), on clinically relevant outcomes of intestinal adaptation, including changes in small-bowel length, small-bowel weight, villus height, and crypt depth in piglets. In this 7-day experiment, delivering only 20% enteral nutrition, we have shown this novel long-acting subcutaneous analogue augments intestinal lengthening and mass, as well as inducing histological adaptation in a distal-intestinal resection neonatal piglet model of SBS. This model is relevant to the developing neonate, wherein growth in intestinal length can be expected to occur both in piglets and humans.[16,17] Furthermore, this is the most relevant model for neonatal SBS given many of the usual causes such as necrotizing enterocolitis and congenital atresia's lead to loss of remnant ileum and ileocecal valve.[25] Finally, using this model, we have previously shown that intestinal adaptation is perturbed in the absence of ileum (associated with limited endogenous GLP-2 secretion), and for this reason, the JC model is particularly useful for testing GLP-2-replacement therapies to enhance intestinal adaptation.[4,9,12]

Using our JC model, we have shown that exogenous GLP-2 replacement, in the form of a continuous intravenous supply of the native analogue, improves both mucosal adaptation and promotes weaning from PN, even in the absence of ileum.[8] A similar benefit of GLP-2 therapy has been confirmed with the subcutaneous analogue teduglutide in piglets[13] and in adult and pediatric humans.[9,10]

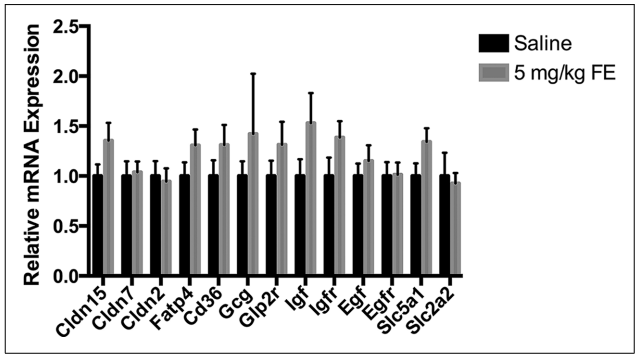

Figure 2. FE 203799 (FE) treatment does not change the expression of intestinal mucosal mRNA. Transcript levels for tight junction proteins, nutrient absorption proteins, enteroendocrine proteins, and enteroendocrine protein receptors from intestinal mucosal scrapes from saline-treated and FE-treated (5 mg/kg) piglets (n = 8–10). CD36, cluster of differentiation-36; CLDN, claudin; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; FATP4, fatty acid transport protein-4; GCG, glucagon gene; GLP2R, glucagon-like peptide 2 receptor; IGF, insulin-like growth factor; IGFR, insulin-like growth factor receptor; SLC5A1, solute carrier family 5 member 1; SLC2A2, solute carrier family 2 member 2.

However, it is notable that in both our own study and that of Thymann et al, also using neonatal piglets, no notable growth in intestinal length was observed with teduglutide. Knowing that the greatest potential for intestinal growth will most likely be observed in the neonatal period of life, it is intriguing that FE induced a marked increase in intestinal length in the current study. Similarly, previous studies in rodents (developmentally mature and using mid-jejunal resection models) have not consistently reported small-intestinal linear growth with GLP-2 treatment.[15,26,27] However, these studies are less relevant for the neonatal human, particularly given that rodents can be maintained on chow alone following massive intestinal resection, and the adaptive response can include cellular hypertrophy, in addition to hyperplasia.[28]

Native GLP-2 differs from teduglutide by 1 single amino acid, and both differ from FE by 3 amino acids; all are 33 amino acids in length. The modified structure of FE differs from teduglutide principally by markedly lower systemic clearance. The half-life of FE makes this peptide poorly recognized by dipeptidyl-peptidase IV, the major determinant of GLP-2 degradation,[29,30] and to exhibit very high protein binding (99.8%). These changes have a functional impact on the pharmacokinetic profile of FE, which differs teduglutide principally by markedly lower systemic clearance (0.6 L/h vs 11 L/j), resulting in an increased half-life. In animals, the half-life of FE is 30 hours compared with 2 hours for teduglutide and only 7 minutes for native GLP-2.

Given that FE-treated piglets had enhanced structural adaptation compared with saline-treated piglets, we might anticipate a reduction in nutrient losses in treated piglets. Although we did confirm significantly lower energy and fat losses per gram of stool in the FE-treated piglets, we did not have a total measurement of wet weight fecal losses per day. As the reduced-spot fecal losses in FE-treated piglets may or may not confer significant clinical advantages, a complete collection and comparison with nutrient delivery is required. However, at this low (trophic) volume of enteral nutrient delivery, there were no real differences in stool output between groups, qualitatively or quantitatively, measured as 24-hour dry stool weight (which was very low). A future direction should be to advance the enteral nutrition well beyond 20% of requirements while simultaneously weaning the PN and determining nutrient absorption, as we have done previously.[8] This would be a better study design to ascertain if the structural differences observed in this study are truly clinically relevant.

There were also no differences in weight gain of the treated piglet's over saline control, again as both groups were pair-fed and received all the nutrient requirements for piglet growth from PN. We do know that teduglutide has been shown to improve nutrient absorption in humans.[31] Prior studies using native GLP-2 in piglets have also shown improved nutrient absorption.[11] Finally, in neonates with SBS, a firm predictor of clinical improvement and weaning off of PN is residual length of the intestine.[4] Therefore, we hypothesize that the observed improved small-intestinal length and structural adaptation with FE in this study should shorten the time required for enteral autonomy. However, this does require confirmation in experimental and clinical studies.

No significant changes were found in either mucosal or whole jejunal mRNA for *Gcg*, *Glp2r*, *Igf-1*, *Igf-1r*, *Egf*, or *Egf-r* following FE treatment. Furthermore, at this level of enteral nutrition, no significant differences were found in nutrient transporter genes, including those for lipid (*Fatp4* and *Cd36*) and carbohydrate (*Slc5a1* and *Scl2a2*) absorption, and of genes for tight junction proteins (*cldn 2*, *cldn 7*, *and cldn 15*). Again, future studies at increased levels of enteral nutrient delivery should repeat these investigations, given that enteral nutrition itself drives intestinal gene expression. Finally, intestinal permeability itself should be explored, given differences have been identified with GLP-2 treatment, and could be done ex vivo using Ussing chambers, as in our prior studies.[32]

Conclusions

In this study, the subcutaneous GLP-2 analogue FE (apraglutide) enhanced intestinal adaptation in a neonatal model of SBS with ileal resection. Compared with our prior experience with continuous GLP-2 in the same piglet

model, when giving only 20% enteral nutrition and studied for only 7 days, the increase in intestinal length noted with FE treatment was unexpected. If such lengthening benefit is further confirmed and is sustained, this would have potential to provide an important clinical benefit for specific treatment of neonatal SBS. We know that residual intestinal length is a key predictor of young children with SBS gaining autonomy from PN.[4] Hence, further studies of FE in neonatal animals are warranted. This should include the demonstration "proof of principle" that the intestinal lengthening observed with treatment does not regress on discontinuation of the treatment itself, as has been observed with the mucosal effects in animal models. Current trials of FE (apraglutide) in adult patients given a once-weekly subcutaneous treatment are underway (NCT 03415594/NCT 03408132), and those clinical outcomes, as well as ongoing preclinical animal studies, will be pivotal to guiding future exploration of this treatment in the pediatric age group.

Acknowledgments

The authors acknowledge the important contribution of Charlane Gorsak and Jette Christiansen for technical expertise and assistance with the study. Therachon has since acquired Glypharma Therapeutics Inc, and the drug now named apraglutide is the property of Therachon.

Statement of Authorship

J. M. Turner, P. W. Wales, and P. L. Brubaker contributed to conception/design of the research; G. M. Slim, M. Lansing, P. Wizzard, P. N. Nation, S. E. Wheeler, P. L. Brubaker, P. B. Jeppesen, and P. W. Wales contributed to acquisition, analysis, or interpretation of the data; G. M. Slim and J. M. Turner drafted the manuscript. All authors critically revised the manuscript, read and approved the final manuscript, and agree to be fully accountable for ensuring the integrity and accuracy of the work.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

References

1. Tappenden KA. Pathophysiology of short bowel syndrome: considerations of resected and residual anatomy. *JPEN J Parenter Enteral Nutr*. 2014;38(1 Suppl):14S-22S.
2. Kosar C, Steinberg K, de Silva N, Avitzur Y, Wales PW. Cost of ambulatory care for the pediatric intestinal failure patient: one-year follow-up after primary discharge. *J Pediatr Surg*. 2016;51(5): 798-803.
3. Khan FA, Squires RH, Litman HJ, et al. Predictors of enteral autonomy in children with intestinal failure: a multicenter cohort study. *J Pediatr*. 2015;167(1):29-34 e1.
4. Belza C, Fitzgerald K, de Silva N, et al. Predicting intestinal adaptation in pediatric intestinal failure: a retrospective cohort study. *Ann Surg*. 2017. [Epub ahead of print].
5. Turner JM, Wales PW, Nation PN, et al. Novel neonatal piglet models of surgical short bowel syndrome with intestinal failure. *J Pediatr Gastroenterolo Nutr*. 2011;52(1):9-16.
6. Hua Z, Turner JM, Sigalet DL, et al. Role of glucagon-like peptide-2 deficiency in neonatal short-bowel syndrome using neonatal piglets. *Pediatr Res*. 2013;73(6):742-749.
7. Brubaker PL. Glucagon-like peptide-2 and the regulation of intestinal growth and function. *Compr Physiol*. 2018;8(3):1185-1210.
8. Suri M, Turner JM, Sigalet DL, et al. Exogenous glucagon-like peptide-2 improves outcomes of intestinal adaptation in a distal-intestinal resection neonatal piglet model of short bowel syndrome. *Pediatr Res*. 2014;76(4):370-377.
9. Carter BA, Cohran VC, Cole CR, et al. Outcomes from a 12-week, open-label, multicenter clinical trial of teduglutide in pediatric short bowel syndrome. *J Pediatr*. 2017;181:102-111.
10. Iyer KR, Kunecki M, Boullata JI, et al. Independence from parenteral nutrition and intravenous fluid support during treatment with teduglutide among patients with intestinal failure associated with short bowel syndrome. *JPEN J Parenter Enteral Nutr*. 2017;41(6):946-951.
11. Vegge A, Thymann T, Lund P, et al. Glucagon-like peptide-2 induces rapid digestive adaptation following intestinal resection in preterm neonates. *Am J Physiol Gastrointest Liver Physiol*. 2013;305(4):G277-G285.
12. Sigalet DL, Brindle ME, Boctor D, et al. A safety and pharmacokinetic dosing study of glucagon-like peptide 2 in infants with intestinal failure. *J Pediatr Surg*. 2017;52(5):749-754.
13. Thymann T, Stoll B, Mecklenburg L, et al. Acute effects of the glucagon-like peptide 2 analogue, teduglutide, on intestinal adaptation in short bowel syndrome. *J Pediatr Gastroenterol Nutr*. 2014;58(6):694-702.
14. Compher C, Gilroy R, Pertkiewicz M, et al. Maintenance of parenteral nutrition volume reduction, without weight loss, after stopping teduglutide in a subset of patients with short bowel syndrome. *JPEN J Parenter Enteral Nutr*. 2011;35(5):603-609.
15. Koopmann MC, Chen X, Holst JJ, Ney DM. Sustained glucagon-like peptide-2 infusion is required for intestinal adaptation, and cessation reverses increased cellularity in rats with intestinal failure. *Am J Physiol Gastrointest Liver Physiol*. 2010;299(6):G1222-G1230.
16. Weaver LT, Austin S, Cole TJ. Small intestinal length: a factor essential for gut adaptation. *Gut*. 1991;32(11):1321-1323.
17. Struijs MC, Diamond IR, de Silva N, Wales PW. Establishing norms for intestinal length in children. *J Pediatr Surg*. 2009;44(5):933-938.
18. Trivedi S, Wiber SC, El-Zimaity HM, Brubaker PL. Glucagon-like peptide-2 increases dysplasia in rodent models of colon cancer. *Am J Physiol Gastrointest Liver Physiol*. 2012;302(8):G840-G849.
19. Middleton G, Stuckey RE. The standardisation of the digestion process in the Kjeldahl determination of nitrogen. *J Pharm Pharmacol*. 1951;3(11):829-842.
20. Englyst HN, Cummings JH. Digestion of polysaccharides of potato in the small intestine of man. *Am J Clin Nutr*. 1987;45(2):423-431.
21. Van De Kamer JH, Ten Bokkel Huinink H, Weyers HA. Rapid method for the determination of fat in feces. *J Biol Chem*. 1949;177(1):347-355.
22. Jeppesen PB, Mortensen PB. Intestinal failure defined by measurements of intestinal energy and wet weight absorption. *Gut*. 2000;46(5):701-706.
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta *C*(*T*)) method. *Methods*. 2001;25(4):402-408.
24. Dube PE, Forse CL, Bahrami J, Brubaker PL. The essential role of insulin-like growth factor-1 in the intestinal tropic effects of glucagon-like peptide-2 in mice. *Gastroenterology*. 2006;131(2):589-605.
25. Squires RH, Duggan C, Teitelbaum DH, et al. Natural history of pediatric intestinal failure: initial report from the Pediatric Intestinal Failure Consortium. *J Pediatr*. 2012;161(4):723-728 e2.

26. Drucker DJ, DeForest L, Brubaker PL. Intestinal response to growth factors administered alone or in combination with human [Gly2]glucagon-like peptide 2. *Am J Physiol*. 1997;273(6 Pt 1): G1252-G1262.
27. Kaji T, Tanaka H, Redstone H, Wallace LE, Holst JJ, Sigalet DL. Temporal changes in the intestinal growth promoting effects of glucagon-like peptide 2 following intestinal resection. *J Surg Res*. 2009;152(2):271-280.
28. Lim DW, Turner JM, Wales PW. Emerging piglet models of neonatal short bowel syndrome. *JPEN J Parenter Enteral Nutr*. 2015;39(6): 636-643.
29. Wisniewski K, Sueiras-Diaz J, Jiang G, et al. Synthesis and pharmacological characterization of novel glucagon-like peptide-2 (glp-2) analogues with low systemic clearance. *J Med Chem*. 2016;59(7):3129-3139.
30. Drucker DJ, Shi Q, Crivici A, et al. Regulation of the biological activity of glucagon-like peptide 2 in vivo by dipeptidyl peptidase IV. *Nat Biotechnol*. 1997;15(7):673-677.
31. Jeppesen PB, Hartmann B, Thulesen J, et al. Glucagon-like peptide 2 improves nutrient absorption and nutritional status in short-bowel patients with no colon. *Gastroenterology*. 2001;120(4):806-815.
32. Lim DW, Levesque CL, Vine DF, et al. Synergy of glucagon-like peptide-2 and epidermal growth factor coadministration on intestinal adaptation in neonatal piglets with short bowel syndrome. *Am J Physiol Gastrointest Liver Physiol*. 2017;312(4): G390-G404.