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Effects of chronic glucagon-like peptide-2 therapy during weaning in neonatal pigs ☆

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ABSTRACT

Background: The enteroendocrine hormone glucagon like peptide-2 (GLP-2) and its ligands are under development as therapeutic agents for a variety of intestinal pathologies. A number of these conditions occur in neonates and infants, and thus a detailed understanding of the effects of GLP-2 during the phase of rapid growth during infancy is required to guide the development of therapeutic applications. We studied the effects of GLP-2 in the neonatal pig to determine the potential effects of exogenous administration.
Methods: Two day old newborn domestic piglets were treated with GLP-2 (1–33) at 40 μg/kg/day or control drug vehicle (saline), by subcutaneous injection, given in two doses per day, (n = 6/group) for 42 days. Animals were weaned normally, over days 21–25. In the fifth week of life, they underwent neuro-developmental testing, and a pharmacokinetic study. On day 42, they were euthanized, and a complete necropsy performed, with histological assessment of tissues from all major organs.
Results: GLP-2 treatment was well tolerated, one control animal died from unrelated causes. There were no effects of GLP-2 on weight gain, feed intake, or behavior. In the treated animals, GLP-2 levels were significantly elevated at 2400 ± 600 pM while at necropsy, organ weights and histology were not affected except in the intestine, where the villus height in the small intestine and the crypt depth, throughout the small intestine and colon, were increased. Similarly, the rate of crypt cell proliferation (Ki-67 staining) was increased in the GLP-2 treated animals and the rate of apoptosis (Caspase-3) was decreased, the depth of the microvilli was increased and the expression of the mRNA for the GLP-2 receptor was decreased throughout the small and large intestine.
Conclusions: In these growing animals, exogenous GLP-2 at pharmacologic doses was well tolerated, with effects confined to the gastrointestinal tract.

1. Introduction

Glucagon like peptide-2 (GLP-2) is a 33 amino acid peptide produced predominantly in the small intestine along with GLP-1 from posttranslational processing of the proglucagon chain in the enteroendocrine L cells [1,2]. Both GLP-2 and GLP-1 are released primarily in response to direct contact with luminal nutrients, especially long-chain fatty acids in the terminal ileum [3]. Although there has been knowledge of the GLP-2 peptide for many years, it was only through studies using pharmacologic doses that the widespread effects on the gastrointestinal tract were discovered [2,4]. GLP-2 has since been shown to induce epithelial proliferation throughout the GI tract, but not in other tissues [1]. Its actions are mediated by a specific G protein coupled receptor, which is expressed in peripheral tissues only in the intestine, in a scattered population of L cells, enteric neurons, and myofibroblasts adjacent to the intestinal crypt zone; giving rise to the specificity of the response to the hormone when given systemically [5–8]. The receptor is also expressed in the central nervous system (CNS), primarily in the hypothalamic region, where it may play a part in the regulation of appetite [9,10]. The trophic effects appear to be mediated by a GLP-2 induced increase in myofibroblast insulin-like growth factor-1 (IGF-1) production which acts as a second messenger to induce intestinal epithelial crypt cell proliferation [11]. The effects of activation of the receptor on enteric neurons may influence the intestinal response

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to inflammation, and the morphology of the enteric nervous system (ENS) itself [8,12]. The expression of the hormone is elevated in premature infants, and may be related to intestinal growth and maturation [13,14]. In animals maintained with parenteral nutrition (PN), GLP-2 monotherapy is sufficient to induce the classical features of intestinal adaptation or the up-regulation of nutrient absorption, which normally requires enteral feeding [15,16]. This suggests that GLP-2 is important in initiating and maintaining spontaneous adaptation, further, exogenous GLP-2 may drive supraphysiologic adaptation [17]. (See Table 1.)

GLP-2 analogues have been developed for use as a therapy in adult patients with intestinal failure, and considerable interest exists in extending this to the relatively large population of infants and children with similar diseases [18,19]. However, before such trials can be conducted, additional study is needed on the effects of pharmacological levels of GLP-2 therapy over the developmental phase of weaning. The present study was developed with the objective of examining the effects of GLP-2 over the phase of intestinal development from suckling, through to weaning. The juvenile pig was chosen as the model, because of the established compatibility of development with the human, and the availability of appropriately sized and nearly genetically identical animals [20]. The primary endpoints were the effects on animal growth, GLP-2 pharmacokinetics, general organ development, and intestinal morphology. In addition, because of the potential for effects on the GLP-2 receptor in the central nervous system (CNS), a behavioral assessment was done. The effect of prolonged exposure to high levels of GLP-2 on the expression of the GLP-2 receptor in the target GI tissues was also quantified. The study was conducted in a University Lab setting, following Good Laboratory Practice (GLP) guidelines.

2. Materials and methods

2.1. Animals and study design

The study protocol was approved by the Animal Care and Use Committee of the University of Alberta, and followed the standards of the Canadian Council on Animal Care. Pregnant inseminated sows (Landrace-Large White cross) were obtained from the Swine Research and Technology Centre (SRTC) of the University of Alberta, Edmonton, Alberta. Sows were housed, fed, and kept under surveillance in the swine facility at the SRTC until parturition.

Twelve pigs from three sows were obtained following spontaneous vaginal birth. Piglets within the normal weight range (1.4 kg–1.8 kg) were randomly assigned to receive either sterile saline (control; n = 6) or GLP-2 injections (40 μg/kg/day, subcutaneously) in two doses (n = 6) 8 h apart starting on the second day of age. The room environment, animal well being, water flow, and feeds were checked twice daily.

2.2. Feeding practices

For twenty-one days, the piglets were kept and fostered with a sow and allowed to nurse sow's milk *ad libitum*. Animals were allowed to move about the protective pen at will. "Creep" feed (an introductory weaning diet, Hi-Pro Feeds, Sherwood Park, AB) was offered in addition to sow's milk, both *ad libitum*, from day-21 of age. After 5 days, the piglets were weaned, and relocated to individual pens, where they were supplied with Starter 2 diet (Hi-Pro Feeds, Sherwood Park, AB) and water *ad libitum*. Thereafter, monitoring of individual feed intake was done daily by the caretakers, feed consumption from the standardized dispenser was measured by weighing residual feed manually twice weekly.

2.3. GLP-2 administration

The dose of GLP-2 used was 40 μg/kg/day, given by s.c. injection twice daily; this dose is twice that used in the majority of adult human trials [21–24]. Human glucagon like peptide 2 (1–33) [26]was produced as a lyophilized powder by the solid state synthetic process (CS Bio, Menlo Park CA) (>98% purity) and prepared as a sterile solution in alkalinized saline (0.9% NaCl alkalinized to pH 8.0–9.0 by addition of.0.05 M NaOH) in individual vials (1.5 mg in 1.5 ml) following good manufacturing practices (GMP) by the experimental therapeutics program of the British Columbia Cancer Agency, Vancouver BC (Lot IC115, May 2009). The peptide was stored as a frozen solution at −20 °C. Stability was demonstrated by repeated testing using an identical mixing and freezing sequence, using mass spectroscopy at 12 and 18 months following the completion of the study, with no loss of protein (Maxxam Corporation, Burnaby BC). The dosing was determined on a weekly basis for each animal based on the measured body weight and adding one half of the body weight gained over the previous week. This method was used to compensate for normal weight gain over each week. The appropriate volumes of compound were prepared in 1 ml syringes, and then refrozen. Individual doses were thawed within 1 h of use; all injections were given using a 0.22 μm filter (Millipore, Etobicoke, Ontario, Canada) and a 23 gauge needle (Becton Dickinson, Mississauga, Ontario, Canada) after aseptic preparation of the skin. Each animal was injected twice daily at 0800 and 1500 h, with the injections rotated between 6 different anatomic sites from day 2 to day 44 of age.

Table 1
Effects of GLP-2 on Gross and Microscopic organ morphology in juvenile pigs. Gross morphology (organ weight, intestinal length and width) of organs as assessed at necropsy Microscopic sections (2 for each organ from each animal) of formalin fixed tissues were stained with hemotoxylin and eosin, and graded by a veterinarian pathologist, blinded as to treatment group. Histological score: 1 = normal, 0.5 = borderline, 0 = abnormal Animals were treated with GLP-2 40 μg/kg/day, given by s.c. injection twice daily (n = 6), and controls with an equivalent volume of saline (n = 5) from day of life 2 to 44. Data: mean ± SEM.

	Control		GLP-2 treated	
Tissue	Gross organ weight/length	Histological score	Gross organ weight/length	Histological score
Stomach (g)	112 ± 13	1.0	107 ± 15	1.0
Liver + gall bladder (g)	587 ± 21	1.0	563 ± 67	1.0
Whole small intestine (g)	787 ± 37	1.0	827 ± 95	1.0
Colon (spiral) (g)	865 ± 55	1.0	1060 ± 129	1.0
Heart (g)	87 ± 3	1.0	91 ± 11	1.0
Lung (g)	189 ± 20	1.0	202 ± 25	1.0
Thymus (g)	36 ± 3	1.0	32 ± 6	1.0
Pancreas (g)	36 ± 3	1.0	33 ± 4	1.0
Spleen (g)	94 ± 5	1.0	86 ± 12	1.0
Kidney (g)	95 ± 4	1.0	89 ± 11	1.0
Brain (g)	60 ± 11	1.0	64 ± 78	1.0
Jejunum (width, cm)	1.7 ± 0.1	1.0	1.9 ± 0.2	1.0
Ileum (width, cm)	1.8 ± 0.1	1.0	1.8 ± 0.2	1.0
Whole small intestine (length, m)	14.1 ± 0.6	1.0	14.9 ± 1.7	1.0