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Is OM-3 synergistic with GLP-2 in intestinal failure?

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ABSTRACT

Introduction: Glucagon-like peptide-2 (GLP-2) is a known intestinal growth factor that enhances mucosal mass and function in residual small intestine after massive small bowel resection (MSBR). Luminal omega-3 (OM-3) has been shown to have some growth factor properties. It is possible that their mechanisms of action differ. Thus, we hypothesized that administering these two substances together may have a synergistic effect.
Methods: A total of 60 adult female Sprague–Dawley rats underwent 80% MSBR and divided as follows (n = 15/group): Saline (Control) + regular feeds; GLP-2 + regular feeds; Saline + OM-3 enriched feeds; and GLP-2 + OM-3 enriched feeds. Five animals per group were sacrificed at 7, 14, and 28 days. Small intestine mucosa was harvested. DNA and protein content were measured (mucosal mass markers) at all three time points. Galactose and Glycine absorption were measured (functional capacity markers) at 28 days. Statistical analysis was done by ANOVA with post hoc Tukey's HSD test.
Results: At all three time points, DNA was increased in all treatment groups compared to control ($P < 0.05$), but GLP-2 + OM-3 group did not have increased DNA content when compared to either treatments alone. At 7 and 14 d, all three treatment groups had increased protein content compared to control ($P < 0.05$). At 28 d, GLP-2 + OM-3 did not have increased protein content compared to control or individual treatments ($P < 1.0$). All three treatment groups had increased absorption of galactose and glycine compared to control ($P < 0.05$) but not each other.
Conclusions: Individually, GLP-2 and OM-3 are very effective in enhancing the adaptive process by increasing mucosal mass and function, at all three time points. More importantly, clinically, GLP-2 and OM-3 increase substrate absorption in a rat model of intestinal failure. However, the combination is not synergistic.

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Introduction

Intestinal failure resulting from massive small bowel loss or the functional loss of intestinal absorptive capacity induces adaptation in the remaining bowel. Extant small intestine dilates and thickens, whereas villous height and crypt depth increase.[1–3] These changes lead to structural adaptation in the form of mucosal hyperplasia, as well as functional increase in nutrient absorption. Rats that have undergone massive small bowel resection (MSBR) as a model of intestinal failure have shown enhanced enteral absorption in the remaining intestine over a 30-d period.[4]

Glucagon-like peptide-2 (GLP-2) is a 33-amino acid peptide[5–7] secreted from enteroendocrine L cells located throughout the intestine, with the highest L-cell concentration in the distal ileum.[8–11] Studies have shown that GLP-2 has a clear trophic effect in the small intestine. Our laboratory has previously evaluated the role of GLP-2 on changes in absorption and histology at various time points during intestinal adaptation.[12–14] These changes result in increased nutrient absorption in the residual small intestine.[15,16] Further studies by our laboratory and others have focused on defining the mechanisms that induce and optimize intestinal adaptation.[17–24]

Omega-3 (OM-3) polyunsaturated fatty acids are metabolized to eicosanoids. It has been shown to have positive effects on bowel adaptation in animal models. In previous studies, OM-3 has been shown to enhance intestinal growth after MSBR and thus may be a growth factor for the intestine. GLP-2 is a known growth factor.[25] It is possible that the mechanism of action for these two substances is different. Thus, our hypothesis was that in combination these two substances may be synergistic in enhancing intestinal absorption and function.

This study was designed to evaluate OM-3 alone and in combination with GLP-2 to determine a potential synergistic effect on mucosal mass, and galactose (carbohydrate) and glycine (amino acid) absorption, as markers of absorption due to intestinal adaptation after MSBR at different time points.

Materials and methods

Experimental model

Sixty young adult female (in this strain, female rats are heartier, less combative) Sprague–Dawley rats weighing between 200 and 250 g were used in this study. The care of these rats was done according to Drexel University Institutional Animal Care and Use Committee regulations under protocol no. 20206. All procedures were done under general anesthesia (intramuscular ketamine 70 mg/kg and xylazine 14 mg/kg) and sterile technique. All rats underwent an approximate 80% small bowel resection leaving 5 cm of jejunum distal to the ligament of Treitz and 5 cm of the ileum proximal to the ileocecal valve, with an end-to-end jejunoileal anastomosis. The rats were equally divided into four groups as follows: group 1 (control, $n = 15$) underwent 80% MSBR with reanastomosis, placement of an internal jugular catheter connected to a subcutaneously placed osmotic minipump (Alzet Osmotic Pumps, Durect Corporation, Cupertino, CA) containing saline and was fed standard rat chow; group 2 (GLP-2; $n = 15$) underwent a 80% MSBR with reanastomosis and osmotic minipump placement containing GLP-2 (Genemed Synthesis Inc, Houston, TX) designed to deliver 100 μg/kg and was fed standard rat chow; group 3 (OM-3, $n = 15$) underwent a 80% MSBR with reanastomosis, placement of an osmotic minipump containing saline and was fed OM-3 (LabDiet, St. Louis, MO) enriched rat chow; group 4 (GLP-2 + OM-3, $n = 15$) underwent a 80% MSBR with reanastomosis, placement of an osmotic minipump containing GLP-2 and was fed OM-3 enriched rat chow. The OM-3 enriched rat chow consisted of 1.2% OM-3 fatty acid. The amount of food consumption was not regulated as the rats consumed diet ad lib. Rats' weights were recorded daily.

Five rats from each group were killed at 7, 14, and 28 d after the pump placement. The remaining small intestine was harvested and was flushed with saline to remove luminal contents, opened, and blotted dry. The ileal mucosa was harvested, placed in RNAlater RNA stabilization reagent (Qiagen, Chatsworth, CA), and snap frozen in liquid nitrogen for further analysis. Rats killed at 28 days underwent absorption studies before euthanization. The absorption studies were done only at 28 d because the intestinal adaptive cycle in rats is completed by this time point. If data were obtained at earlier time points that showed possible synergy but was not sustained at 28 d (evident at the end of adaptive process), it would not be clinically relevant.

Of note, we have not performed histology for many years because the parameters of mucosal DNA and protein used in our study are more accurate and less subjective than histology.

DNA extraction and quantitation

Mucosal samples were thawed, blotted dry, and weighed. DNA was extracted using the Gentra Puregene tissue kit (Qiagen, Germantown, MD). Mucosal DNA content was measured at 260 nm with purity measured by absorbance ratio 260 nm/280 nm using a Beckman–Coulter DU 640 spectrophotometer. Samples with 260 nm/280 nm ratio <1.7 or >1.9 were discarded as impure. Concentration was then normalized to micrograms DNA per milligram mucosa (μg/mg).

Protein extraction and quantitation

Mucosal samples were thawed, blotted dry, and weighed. Each sample was then individually placed in a test tube with 1 × phosphate-buffered saline (Mediatech, Inc, Herndon, VA) and homogenized. The sample was then centrifuged at 3000 g for 10 min. After centrifugation, the supernatant was discarded, and 1 mL of Solulyse-M (Genlantis, San Diego, CA) was added, and the sample was shaken for 10 min. The sample was again centrifuged for 15 min at 3000 g. A 20-μL sample of supernatant was transferred to three semimicro cuvettes (Bio-Rad, Hercules, California) per animal sample. One milliliter of room temperature Quick Start Bradford Dye Reagent, 1 × (Bio-Rad), was added to each semimicro cuvette and mixed with a

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