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# Hepatocyte growth factor and omega-3–enriched feeds have a synergistic effect on mucosal mass in an animal model of inflammatory bowel disease

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**Abstract**

**Background:** Hepatocyte growth factor (HGF) decreases intestinal inflammation and cytokine levels in an animal model of inflammatory bowel disease (IBD). Luminal omega-3 (OM-3) is anti-angiogenic, reduces inflammation, and may decrease symptoms in patients with Crohn's disease. This study evaluates the synergism of HGF and OM-3.

**Methods:** Twenty adult female transgenic HLA-B27 rats were divided into 4 groups: group 1: regular feeds, IV saline; group 2: OM-3–enriched feeds, IV saline; group 3: regular feeds, IV HGF (150 μg/kg per day); and group 4: OM-3–enriched feeds, IV HGF(150 μg/kg per day). Rats were killed at 14 days after pump placement. Small and large bowel mucosa was harvested, and DNA and protein were extracted and quantified. Statistical analysis was done by analysis of variance with post-hoc Tukey's HSD test.

**Results:** Content of protein and DNA in the ileum were significantly increased by supplementation of HGF ($P < .001$, $P < .01$, respectively) alone. OM-3 significantly increased protein content but not DNA ($P = .02$, $P = 0.3$, respectively). Combined, they had a synergistic effect greater than either supplement alone ($P = .0001$, $P = .002$, respectively). In the colon, HGF and OM-3 did not significantly increase protein or DNA content individually or together.

**Conclusions:** This is the first demonstration of the synergistic effect of a growth factor (HGF) and a dietary supplement (OM-3) in an immunologic model of IBD.



Inflammatory bowel disease (IBD) is a chronic, relapsing, and often unrelenting inflammatory disease affecting the small and large bowel, which can lead to intestinal failure. The 2 primary disease processes associated with IBD are ulcerative colitis and Crohn's disease. Despite extensive research, the etiology remains unknown. Management of IBD involves treatments that aim to diminish the inflammatory nature of the disease.

Using an immunologic animal model of IBD, our laboratory has previously investigated the potential benefit of growth factors in the management of IBD. Specifically, we have evaluated clinical parameters, gross appearance,

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histology, intestinal angiogenesis, inflammatory markers, and apoptosis. The purpose of this study was to determine if the known effects of HGF in this model would be enhanced by enteral omega-3 (OM-3). Fisher rats transfected with the HLA-B27 gene develop spontaneous chronic gastrointestinal (GI) inflammation by 20 weeks of age under pathogen-specific conditions. The lesions are similar to those seen in patients with IBD. Using this immunologic animal IBD model, our laboratory has previously shown that hepatocyte growth factor (HGF) can substantially ameliorate the gross and microscopic changes of colitis seen in this IBD model, as well as microscopic inflammatory score [1], increase microvascular density in the ileum and colon, decrease inflammatory cell migration, decrease inflammatory mediators (IL-6 and TNF-α), and improve ulceration and hemorrhage of the mucosa seen on histology [2]. Regarding the DNA content, this is a direct correlation to mucosal mass, which is an increase in villous height or villous density. First discovered in 1984 [3], and later purified from rat platelets in 1986 [4], HGF is a heterodimer composed of a 69-kd α subunit and 34-kd β subunit [5]. It is a pleiotrophic growth factor secreted from mesenchymal cells that target the C-Met receptor. This receptor, which has tyrosine kinase activity, was first identified in 1991 [6]. It is found in several organs including the intestine, along with the central nervous system, lung, kidney, and liver [7].

Omega-6 and omega-3 polyunsaturated fatty acids are metabolized to eicosanoids, which play an important role in the regulation of inflammation. Eicosanoids derived from omega-6 polyunsaturated fatty acids have proinflammatory and immunoactive functions [8], whereas eicosanoids derived from OM-3 polyunsaturated fatty acids have anti-inflammatory properties, mostly because of the competitive inhibition of omega-6–derived eicosanoids [9]. Luminal OM-3 has been shown to be anti-angiogenic [10], increase apoptosis [11], down-regulate PGE-2 and COX-2 in colonic mucosa [12], reduce inflammation [13], and may be protective against Crohn's disease [14]. This study was designed to evaluate the potential synergistic effect of HGF and OM-3.

## 1. Materials and methods

### 1.1. Animal model

All storage, handling, and procedures were done following the guidelines for animal experimentation of the University Institutional Animal Care and Use Committee and approved under protocol no. 17714. Female adult Fisher rats transfected with the HLA-B27 gene (Taconic Farms, Hudson, NY) were housed in cages in groups of 3 at a constant temperature (22°C) with 12-hour light-dark cycles until 20 weeks of age. Throughout this period, half of the rats were fed a standard laboratory chow (Lab Diet; Purina, Richmond, Ind), and the other half were fed an OM-3–enriched diet (Mod TestDiet® AIN-93G/Custom Fatty Acid Content; TestDiet, Richmond, IN), which contains 4× the OM-3 fatty acid content found in the standard rat chow. All rats were given water ad libitum and maintained in conditions as recommended by the source (Taconic Farms, Hudson, NY).

### 1.2. Experimental model

Twenty adult female transgenic HLA-B27 rats weighing between 200 and 250 g were used for this study. Procedures were done under general anesthesia (intraperitoneal ketamine 70 mg/kg and xylazine 14 mg/kg) and sterile technique. The rats were equally divided into 4 groups based on the type of rat chow they were fed beginning with arrival at the animal facility (with or without enriched OM-3) and the content of their subcutaneously placed osmotic minipump (Alza, Palo Alto, CA) (with or without recombinant human HGF 150 mg/kg per day reconstituted in 20 mmol/L Tris-HCl [pH 7.5] and 1% PBS, Genentech, San Francisco, Calif). The subcutaneous minipumps were placed 20 weeks after arrival at the animal facility when the rats developed colitis and are designed to deliver their contents over 14 days at a rate of 0.5 uL/h. The 4 groups are as follows: group 1 (control, n = 5) underwent placement of an internal jugular catheter connected to a subcutaneously placed osmotic minipump containing saline and were fed regular rat chow; group 2 (OM-3, n = 5) underwent placement of an internal jugular catheter connected to a subcutaneously placed osmotic minipump containing saline and were fed OM-3–enriched feeds; group 3 (HGF, n = 5) underwent placement of an internal jugular catheter connected to a subcutaneously placed osmotic minipump containing HGF at 150 μg/kg per day and were fed regular rat chow; group 4 (HGF&OM-3, n = 5) underwent placement of an internal jugular catheter connected to a subcutaneously placed osmotic minipump containing HGF at 150 μg/kg per day and were fed OM-3–enriched feeds. Postoperatively, the rats were given their respective feeds and water ad libitum. All rats were killed at 14 days after pump placement. Small and large bowel mucosa was harvested, flushed with saline to remove luminal contents, opened, and blotted dry. The mucosa was harvested and snap frozen in liquid nitrogen for further analysis. The remaining bowel was grossly inspected to verify complete mucosal harvesting.

### 1.3. 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 minutes. After centrifugation,