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Hepatocyte growth factor, hepatocyte growth factor activator and arginine in a rat fulminant colitis model

Nathan P. Zwintscher [a], Puja M. Shah [b], Shashikumar K. Salgar [c], Christopher R. Newton [a, d], Justin A. Maykel [e], Ahmed Samy [f], Murad Jabir [f], Scott R. Steele [f, *]

[a] Department of Surgery, Madigan Army Medical Center, Tacoma, WA, USA
[b] Department of Surgery, University of Virginia, Charlottesville, VA, USA
[c] Department of Clinical Investigation, Madigan Army Medical Center, Tacoma, WA, USA
[d] Department of Surgery, Children's Hospital & Research Center Oakland, Oakland, CA, USA
[e] Department of Surgery, UMass Memorial Medical Center, Worcester, MA, USA
[f] Division of Colon and Rectal Surgery, University Hospitals Case Medical Center, Cleveland, OH, USA

HIGHLIGHTS

- We developed a fulminant colitis model in adolescent rats.
- The fulminant colitis model reproduces inflammatory bowel disease in humans.
- The rats were treated with hepatocyte growth factor, its activator, and arginine.
- The HGF treated rats had fewer days of pain.
- The arginine treated rats had fewer days of diarrhea.

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ABSTRACT

Introduction: Dextran sodium sulfate (DSS) is commonly used to induce a murine fulminant colitis model. Hepatocyte growth factor (HGF) has been shown to decrease the symptoms of inflammatory bowel disease (IBD) but the effect of its activator, HGFA, is not well characterized. Arginine reduces effects of oxidative stress but its effect on IBD is not well known. The primary aim is to determine whether HGF and HGFA, or arginine will decrease IBD symptoms such as pain and diarrhea in a DSS-induced fulminant colitis murine model.

Methods: A severe colitis was induced in young, male Fischer 344 rats with 4% (w/v) DSS oral solution for seven days; rats were sacrificed on day 10. Rats were divided into five groups of 8 animals: control, HGF (700 mcg/kg/dose), HGF and HGFA (10 mcg/dose), HGF and arginine, and high dose HGF (2800 mcg/kg/dose). Main clinical outcomes were pain, diarrhea and weight loss. Blinded pathologists scored the terminal ileum and distal colon.

Results: DSS reliably induced severe active colitis in 90% of animals ($n = 36/40$). There were no differences in injury scores between control and treatment animals. HGF led to 1.38 fewer days in pain ($p = 0.036$), while arginine led to 1.88 fewer days of diarrhea ($P = 0.017$) compared to controls. 88% of HGFA-treated rats started regaining weight ($P < 0.001$).

Discussion/Conclusion: Although treatment was unable to reverse fulminant disease, HGF and arginine were associated with decreased days of pain and diarrhea. These clinical interventions may reduce associated symptoms for severe IBD patients, even when urgent surgical intervention remains the only viable option.

* Corresponding author. Madigan Army Medical Center, Department of Surgery, Tacoma, WA 98431, USA.
E-mail addresses: Ahmedsamy.md@gmail.com (A. Samy), muradjab2010@gmail.com (M. Jabir), harkersteele@mac.com (S.R. Steele).

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1. Introduction

The continuum of inflammatory bowel disease (IBD) includes Crohn's disease (CD), Ulcerative Colitis (UC) and indeterminate colitis. In adults and children alike, patients tend to present with abdominal pain, diarrhea, rectal bleeding, weight loss, and perforation [1,2]. Children carry the additional burden of potential growth retardation in moderate to severe UC [3].

Goals of management are to control or minimize disease by suppressing inflammation and immune response modulation. Despite this, approximately 15–30% of patients undergo subtotal colectomy with end ileostomy due to disease progression, failure of medical therapy or personal preference. Another 20–30% of patients with fulminant colitis require urgent surgery [4–7]. Although the focus of medical therapy for UC has been on suppression of inflammation, improved understanding is needed about the potential effects of growth factors geared at the mucosa in IBD.

Hepatocyte growth factor (HGF) is a heterodimer secreted by mesenchymal cells found in the liver, lung, central nervous system and the intestine and enhances epithelial cell proliferation in murine models [8]. HGF is known to decrease IBD symptomatology and lowers intestinal injury scores in IBD rat models [9,10]. It has additional benefits of reducing inflammatory pathways and apoptosis in liver injury [11] and ischemia-reperfusion models [12]. Conversely, the effect of its activator, hepatocyte growth factor activator (HGFA), is not well delineated. Supplementing its activator should theoretically increase the effect of HGF and fulminant colitis recovery. Finally, arginine (ARG) is an amino acid that has protective effects against oxidative stress in healthy newborn rats, hypoxia-reoxygenation, exercise and diabetes [13–15]. It likely exerts effects via inducible nitric oxide synthase, which may increase epithelial wound repair [16]. Consequently, it may protect intestinal mucosa by ameliorating the inflammatory response seen so commonly amongst IBD patients.

We hypothesized clinical and pathologic improvement in colonic and terminal ileum mucosa in an experimental rat model of fulminant colitis versus control animals, by supplementing HGF treatment with HGFA and arginine. We also expected that HGF plus HGFA would synergistically promote earlier recovery from severe acute colitis in the rodent model. Primary endpoints included symptoms such as pain and diarrhea.

2. Materials and methods

2.1. Animal model

Following Institutional Animal Care and Use Committee approval (local IACUC protocol 210084), an active, severe colitis was induced in 40 young, male Fischer 344 rats (8–12 weeks of age) with a 4% (w/v) DSS oral solution for seven days via standard water bottles. The DSS solution was the water source for the animals during the period of injury. 40 rats were divided equally into 5 groups by treatment regimen. Group 1 (control, n = 8) was untreated and was a historical control; group 2 (HGF, n = 8) received 700 mcg/kg/dose of HGF (Genentech, South San Francisco, CA) subcutaneously on days 5, 7 and 9; group 3 (HGF+ARG) was given 700 mcg/kg/dose of HGF and administered arginine as a 1% (w/v) oral solution on days 5–10; group 4 (HGF+HGFA) was given 700 mcg/kg/dose of HGF and also given 10 mcg/dose of HGFA (human recombinant HGF activator, R&D Systems, Minneapolis, MN) subcutaneously on days 5 and 7; group 5 (HGFx4, high dose HGF) was given 2800 mcg/kg/dose of HGF. 700 mcg/kg/dose of HGF was chosen to mirror the total HGF dose of a prior model [10]. HGFA dosing was selected based on commercially available quantities, since there is no published data for HGFA dosing. Treatment started on day 5 based on our prior work that demonstrated the rats would have an ongoing active colitis. All rats were sacrificed on day 10 to allow for potential recovery after DSS injury ended on day 7. This would allow for five total days of treatment and corresponds to earlier surgical intervention, which is associated with improved postoperative outcomes [17]. This timing also corresponds to the point at which patients with steroid refractory UC should be considered for colectomy (5–7 days) [18]. One animal in the arginine group was sacrificed on day 9 due to significant pain, weight loss, lethargy and overall appearance.

2.2. Clinical monitoring

Rats were housed in standard polycarbonate individual caging systems. They were allowed access to standard rat chow and water ad libitum. On days 1–7, DSS was added to the water source in a 4% concentration (w/v) [16]. Similarly for HGF+ARG group, the water source also included arginine (1% w/v) on days 5–10 [16].

A veterinarian and veterinarian technologists closely monitored the rats. They were observed for overall health and their appearance (hunched, not groomed), presence of diarrhea (Type 6 or 7 on Bristol Stool Scale) [19] or gross blood, and need for pain medication was recorded. Rats were determined to be in pain if they maintained a hunched position or a ruffled, unkempt coat [20] and had binary documentation. If rats were deemed to be in pain on at least one of the twice-daily checks they were marked as being in pain for the day. Similarly, rats were marked as having diarrhea for the day if a single stool was diarrhea. Buprenorphine (0.01 mg/dose) was administered subcutaneously as necessary for pain control.

2.3. Histopathology

Rats were euthanized with carbon dioxide at time of necropsy. A midline laparotomy was used to harvest the similar segments of distal colon and distal ileum. The specimens were stored in 10% formalin, and delivered to two blinded pathologists. The small bowel was graded on a scale from 0 to 8 (0 = normal mucosa; 1 = subepithelial space at villus tip; 2 = extended subepithelial space; 3 = epithelial lifting along villus sides; 4 = denuded villi; 5 = loss of villus tissue; 6 = crypt layer infarction; 7 = transmucosal infarction; 8 = transmural infarction) [21]. The colon samples (Fig. 1) were graded on a scale from 0 to 3 (0 = no inflammation; 1 = mild inflammation with cryptitis; 2 = moderate inflammation with crypt abscesses; 3 = severe inflammation with crypt abscesses and surface erosion or ulceration) [10]. Injury scores were averaged between the two pathologists.

2.4. Statistical analysis

Analysis of variance (ANOVA) with Bonferroni correction was used to analyze the rats' appearance, pain, character of stool and percent weight loss. Binary logistic regression was used to determine if rats started to regain weight. ANOVA with Bonferroni correction was also used to analyze the rats' colon and small bowel injury scores. Statistical significance was set at $P < 0.05$.

2.5. Ethical considerations

Research on animals in this study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the National Research Council, 2010. This study was approved by the local Institutional Review Board and the Institutional Animal Care and Use Committee (IACUC) at the Madigan Army Medical