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Forced treadmill exercise training exacerbates inflammation and causes mortality while voluntary wheel training is protective in a mouse model of colitis

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ABSTRACT

The purpose of this study was to examine whether exercise training reduced inflammation and symptomology in a mouse model of colitis. We hypothesized that moderate forced treadmill running (FTR) or voluntary wheel running (VWR) would reduce colitis symptoms and colon inflammation in response to dextran sodium sulfate (DSS). Male C57Bl/6J mice were randomized to sedentary, moderate intensity FTR (8–12 m/min, 40 min, 6 weeks, 5x/week), or VWR (30 days access to wheels). DSS was given at 2% (w/v) in drinking water over 5 days. Mice discontinued exercise 24 h prior to and during DSS treatment. Colons were harvested on Days 6, 8 and 12 in FTR and Day 8 post-DSS in VWR experiments. Contrary to our hypothesis, we found that moderate FTR exacerbated colitis symptomology and inflammation as measured by significant ($p < 0.05$) increases in diarrhea and IL-6, IL-1β, IL-17 colon gene expression. We also observed higher mortality (3/10 died vs. 0/10, $p = 0.07$) in the FTR/DSS group. In contrast, VWR alleviated colitis symptoms and reduced inflammatory gene expression in the colons of DSS-treated mice ($p < 0.05$). While DSS treatment reduced food/fluid intake and body weight, there was a tendency for FTR to exacerbate, and for VWR to attenuate, this effect. FTR (in the absence of DSS) increased gene expression of the chemokine and antibacterial protein CCL6 suggesting that FTR altered gut homeostasis that may be related to the exaggerated response to DSS. In conclusion, we found that FTR exacerbated, whereas VWR attenuated, symptoms and inflammation in response to DSS.

1. Introduction

Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC), cause chronic morbidity that significantly reduce physical functioning and quality of life in afflicted patients. While UC is an idiopathic disorder, it is clear that environmental (e.g. infection, diet, psychological stress) and host genetic factors, in combination with disturbances in the intestinal microbiome, trigger barrier disruption leading to dysregulated inflammation in response to resident gut bacteria (Sanchez-Munoz et al., 2008). Immune cell infiltration stimulates the production of pro-inflammatory cytokines, which promote a chronic inflammatory state, instigating clinical symptoms including colon ulcers, rectal bleeding, diarrhea, abdominal pain, fatigue, and an overall altered emotional well-being (Sanchez-Munoz et al., 2008). Furthermore, UC significantly increases the risk of developing colorectal cancer later in life (Rizzo et al., 2011).

It is well established that anti-inflammatory therapies, designed to decrease colon inflammation, attenuate the symptoms of UC in humans and mouse models of the disease (Bi and Triadafilopoulos, 2003). Anti-inflammatory pharmaceutical treatments, such as 5-aminosalicylic acid and corticosteroids, are beneficial in reducing symptoms, but their efficacy is not complete and they have significant side-effects. Therefore, in addition to more efficacious and safer drug development, investigation of adjunct anti-inflammatory therapies that could attenuate colitis and counteract side effects of conventional treatment is of prime importance from a public health perspective. Such strategies could assist in improving the quality of life by reducing symptoms, hospitalizations, and the risk of colorectal cancer in UC patients.

Exercise has been suggested as an adjunct anti-inflammatory therapy for chronic diseases associated with inflammation (Astrom et al., 2010). Indeed, our laboratory as well as others, have provided

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evidence that moderate treadmill exercise acts in an anti-inflammatory fashion and bolsters immunity in humans (Vieira et al., 2009a; Packer et al., 2010; Walsh et al., 2011) and in animals where exaggerated or chronic inflammation exists (Lowder et al., 2006; Keylock et al., 2008; Vieira et al., 2009b). With respect to the gut, while there is significant evidence that regular moderate exercise can reduce risk for colon cancer, there are very few studies on whether it can exert positive effects in people with IBD. However, there is evidence that prolonged, intense exercise bouts can cause debilitating gastrointestinal complaints including cramps, diarrhea and gut inflammation most likely due to gut ischemia and/or mechanical trauma (Bi and Triadafilopoulos, 2003; Packer et al., 2010). Results from the few observational studies of exercise and IBD reveal that regularly performed moderate exercise neither protects from nor initiates IBD (Narula and Fedorak, 2008). However, definitive randomized exercise trials are lacking.

When given in the drinking water to rodents, DSS induces reproducible lesions in the distal colon by interfering with barrier function and stimulating local inflammation mimicking the pathophysiology of human colitis (Laroui et al., 2012). Although models such as DSS-induced colitis do not represent the complexity of the human disease, investigating intestinal inflammation in animal models has produced valuable data that have been useful in uncovering signaling pathways during colon inflammation (Fukata et al., 2005), the cell types and cytokines responsible for disease activity (Elrod et al., 2005), and the local and systemic pathophysiology of acute and chronic colitis (Mizoguchi et al., 2003; Wirtz et al., 2007; Hamdani et al., 2008). Additionally, these models are valuable and indispensable tools for manipulating factors that affect pathogenesis and evaluating potential therapeutic strategies (Wirtz et al., 2007). Therefore, given the lack of knowledge in this area, the purpose of our study was to investigate the effect of moderate forced treadmill and voluntary wheel running on DSS-induced colitis mortality, morbidity, colon inflammation and colon histopathology in mice. We hypothesized that exercise would reduce symptoms and inflammatory burden in the colon in response to DSS.

2. Methods

2.1. Animals

Eight to ten week old male C57Bl/6J mice (forced treadmill run [FTR], $n = 132$; and voluntary wheel run [VWR], $n = 26$) were purchased from Jackson Laboratories (Bar Harbor, ME) and singly housed in an accredited specific pathogen-free (SPF) barrier facility. Animals were housed under a reverse light-dark cycle (lights on at 9 PM, lights off at 9 AM) in a low stress environment and given *ad libitum* access to sterile water (unless on study) and autoclaved rodent chow. Mice were acclimated to the facility for 1 week prior to the experiments. All corncob bedding and cages were sterilized before use and were changed weekly. All experiments were approved by the University of Illinois Urbana-Champaign IACUC. Three separate FTR experiments were performed with euthanasia occurring at Day 6 ($n = 10$/group), Day 8 ($n = 13$/group) or Day 12 ($n = 10$/group) post-DSS treatment. The VWR experiment was performed with euthanasia occurring at Day 8 post-DSS at the time of peak symptoms (control: $n = 5$/group; DSS: $n = 8$/group).

2.2. Exercise protocol

For the FTR experiments, mice were paired on body weight prior to training or sedentary control conditions and then randomized into one of four groups; sedentary water (SED/H_2O), sedentary DSS (SED/DSS), exercised water (FTR/H_2O) and exercised DSS (FTR/DSS). Mice (including exercise trained mice) did not run during or after DSS treatment to minimize distress. Using this design, we were interested in testing the effects of prior exercise training on response to DSS-induced colitis. FTR mice performed 6 weeks (5 days/week: total 30 sessions) of forced moderate treadmill running (8–12 m/min; 5% grade; equating to ~480 m of running per session) for 40 min per day at the beginning of their dark cycle prior to DSS treatment. For the VWR experiment, mice were housed in cages with free access to telemetered running wheels (Respironics, Bend, OR) for 30 days. Thus, we controlled for the number of days of exposure to exercise, but not exercise intensity or volume between the two different exercise paradigms. The last exercise session occurred 24 h prior to DSS administration. All sedentary mice were handled similarly and housed in close proximity to the treadmill and wheel cages during the exercise training to control for incidental stress associated with handling, noise and novel environmental exposure.

2.3. DSS treatment

Regular drinking water was replaced by 2% DSS in sterile water (w/v, MP Biochemical 36,000–50,000 MW) for a period of 5 days after completion of the exercise intervention period. The DSS solution was monitored daily, refilled appropriately and replaced on Day 3.

2.4. Morbidity

Daily measurements of body weight and food and fluid intake were made during and after DSS administration. In addition, mouse feces were evaluated in an observer blinded manner for consistency/diarrhea (e.g. formed pellet vs. semi-formed/soft) and were tested daily for the presence of blood (Hemoccult®). Lastly, we assessed the physical activity behavior (e.g. 1 = 'normal, 2 = 'slight reduction', 3 = 'limited', 4 = 'immobile') in the home cage and response to capture (e.g. 1 = 'normal evasion', 2 = 'some evasion', 3 = 'no evasion') as indicators of sickness behavior in a blinded manner.

2.5. Tissue collection

Mice were euthanized by rapid CO_2 asphyxiation and cervical dislocation. Blood was drawn from the inferior vena cava and stored on ice. Plasma was separated and stored at −80 °C. Mice were then perfused via intra-cardial injection of sterile saline. The colon was removed (distal to the caecum to the anus) and colon length was assessed using digital calipers (Tresna) to the nearest mm. Mesentery tissue was removed from the colon which was flushed with PBS and separated into proximal and distal ends. A 2 cm piece was cut from the top portion of the distal half and fixed in 10% buffered formalin until sectioning for histological analysis. Proximal and distal ends were stored at −80 °C until analysis. Both adrenals and the thymus were excised and weighed to screen for adrenal hypertrophy and thymic involution.

2.6. Colon histological analysis

The 2 cm long 10% formalin fixed colon samples were paraffin embedded and 3 μm cross sections were stained with hematoxylin and eosin. Colon damage (necrosis) and immune cell infiltrate was scored by a blinded observer based on a published scoring system that considers architectural derangements, goblet cell depletion, edema/ulceration, and degree of inflammatory cell infiltrate (Wirtz et al., 2007). Each sample was ranked twice, once for necrosis and once for inflammation. Scoring for inflammatory cell infiltration was as follows: 0 = 'normal', 1 = 'mild', 2 = 'moderate', 3 = 'severe'.