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Minocycline attenuates experimental colitis in mice by blocking expression of inducible nitric oxide synthase and matrix metalloproteinases

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ABSTRACT

In addition to its antimicrobial activity, minocycline exerts anti-inflammatory effects in several disease models. However, whether minocycline affects the pathogenesis of inflammatory bowel disease has not been determined. We investigated the effects of minocycline on experimental colitis and its underlying mechanisms. Acute and chronic colitis were induced in mice by treatment with dextran sulfate sodium (DSS) or trinitrobenzene sulfonic acid (TNBS), and the effect of minocycline on colonic injury was assessed clinically and histologically. Prophylactic and therapeutic treatment of mice with minocycline significantly diminished mortality rate and attenuated the severity of DSS-induced acute colitis. Mechanistically, minocycline administration suppressed inducible nitric oxide synthase (iNOS) expression and nitrotyrosine production, inhibited proinflammatory cytokine expression, repressed the elevated mRNA expression of matrix metalloproteinases (MMPs) 2, 3, 9, and 13, diminished the apoptotic index in colonic tissues, and inhibited nitric oxide production in the serum of mice with DSS-induced acute colitis. In DSS-induced chronic colitis, minocycline treatment also reduced body weight loss, improved colonic histology, and blocked expression of iNOS, proinflammatory cytokines, and MMPs from colonic tissues. Similarly, minocycline could ameliorate the severity of TNBS-induced acute colitis in mice by decreasing mortality rate and inhibiting proinflammatory cytokine expression in colonic tissues. These results demonstrate that minocycline protects mice against DSS- and TNBS-induced colitis, probably via inhibition of iNOS and MMP expression in intestinal tissues. Therefore, minocycline is a potential remedy for human inflammatory bowel diseases.

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Introduction

Human inflammatory bowel disease (IBD) is an idiopathic and chronic inflammation of the gastrointestinal tract (Korzenik and Podolsky, 2006), and 20% to 40% of patients with IBD also exhibit extraintestinal manifestations involving the joints, skin, eyes, or hepatobiliary tract (Barrie and Regueiro, 2007). The pathogenesis of IBD involves a dysregulated immune response and consequent activation of inflammatory cascades, which are often affected by genetic susceptibility and environmental factors such as intestinal commensal bacteria or their products (Bouma and Strober, 2003; Macdonald and Monteleone, 2005; Korzenik and Podolsky, 2006).

Most conventional IBD therapies rely primarily on downregulating aberrant immune responses and inflammatory cascades (Bouma and Strober, 2003; Korzenik and Podolsky, 2006). Mesalamine and corticosteroids are the mainstays of treatment for acute forms of IBD. However, 20% to 32% of IBD patients do not respond to steroid therapy (Truelove and Witts, 1955; Munkholm et al., 1994). Some antibiotics offer protection against experimental and clinical colitis (Onderdonk et al., 1978; Rath et al., 2001; Sartor, 2004); intestinal inflammation does not occur when at-risk mice are kept in a germ-free environment (Sadlack et al., 1993; Sellon et al., 1998). This indicates that the gut microflora plays an accessory but indispensable role in the progression of IBD. However, the clinical use of metronidazole and/or ciprofloxacin is only effective against certain types of Crohn's disease and remains a controversial treatment for ulcerative colitis (Sartor, 2004). Alternative treatments involving the use of immunosuppressants and tumor necrosis factor-α (TNF-α) antagonists have been associated with significant complications and

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require cautious application during long-term therapy (Keane et al., 2001; Warris et al., 2001).

Nitrosative stress caused by inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO) production is strongly associated with the progression of IBD and contributes to the pathogenesis of human IBD and experimental colitis (Boughton-Smith, 1994; Cross and Wilson, 2003). Blockade of iNOS expression using gene knockout or specific inhibitors ameliorates the severity of experimental colitis (Zingarelli et al., 1999; Hokari et al., 2001; Krieglstein et al., 2001). In addition, matrix metalloproteinases (MMPs) have been shown to play an important role in the pathogenesis of IBD (Baugh et al., 1999; Medina and Radomski, 2006). As in human IBD, expression of MMPs is also implicated in the pathogenesis of experimental colitis, as MMP blockade by genetic or pharmacologic inhibition attenuates the severity of experimental colitis induced by dextran sulfate sodium (DSS) or trinitrobenzene sulfonic acid (TNBS) (Medina et al., 2003; Naito et al., 2004; Castaneda et al., 2005; Medina et al., 2006). Therefore, it is important to develop and evaluate agents that have low-severity adverse effects that can treat IBD by blocking inflammatory responses involved in nitrosative stress and/or MMP activation.

Minocycline, a semisynthetic tetracycline, is a safe, widely used and inexpensive antibiotic with a broad spectrum. Several recent studies have demonstrated that, in addition to its antimicrobial effects, minocycline exerts anti-inflammatory, antiangiogenic, and antiapoptotic effects. These biological effects of minocycline have been shown to have a therapeutic or preventive effect in neurodegenerative disease (Du et al., 2001; Thomas et al., 2004), neural ischemic damage (Koistinaho et al., 2005), ischemic renal injury (Sutton et al., 2005), rheumatoid arthritis (Stone et al., 2003), acne vulgaris (Sapadin and Fleischmajer, 2006), pyoderma gangrenosum (Shenefelt, 1996), and periodontitis (Rifkin et al., 1993). The mechanisms by which minocycline alleviates these illnesses involve suppressing expression and/or activity of iNOS (Amin et al., 1996; Amin et al., 1997), MMPs (Rifkin et al., 1993; Lee et al., 2006; Machado et al., 2006), TNF-α (Sriram et al., 2006) and caspases (Chen et al., 2000), and blocking cytochrome-*c* release (Zhu et al., 2002; Chu et al., 2005).

Several animal models of intestinal inflammation have been established, although some only partially resemble human IBD (Elson et al., 1995; Wirtz and Neurath, 2000). The murine model of DSS-induced acute or chronic colitis is well established and widely used (Okayasu et al., 1990; Elson et al., 1995) and is characterized by direct mucosal/submucosal damage, which is particularly severe in the distal colon and thus mimics human ulcerative colitis. TNBS-induced colitis in mice, which is typified by colonic transmural damage caused by hapten-induced delayed hypersensitivity, has been used as a model to study human Crohn's disease (Morris et al., 1989; Elson et al., 1995). In this study, we used these two murine colonic damage models to examine the effects of minocycline treatment and found that minocycline significantly diminished the severity of DSS- and TNBS-induced colitis, probably via its anti-inflammatory and antimicrobial effects.

Materials and methods

Animals. Male C57BL/6J and BALB/c mice (7–9 weeks old) were purchased from the National Laboratory Animal Center (Taipei, Taiwan) and housed in the laboratory animal center of the National Defense Medical Center (NDMC), Taipei. All studies adhered to the Declaration of Helsinki and the institutional guidelines of the NDMC.

DSS-induced acute and chronic colitis. C57BL/6J mice were used to study DSS-induced colitis (Mahler et al., 1998). This strain is susceptible to DSS assault and has been widely used as a mouse colitis model. Acute colitis was induced in C57BL/6J mice by adding DSS (35–45 kDa, TdB Consultancy, Uppsala, Sweden) to the drinking water at a level of 3% for a period of 5 days, after which the mice received regular tap water. Body weight loss, stool consistency, and blood in the stool were monitored daily to assess the severity of colitis as previously described (Cooper et al., 1993). Weight loss was arbitrarily scored as 1, 1%–5%; 2, 5%–10%; 3, 10%–15%; and 4, >15%. Blood in the stool was scored as 0, normal; 2, slight bleeding; and 4, gross bleeding. Diarrhea was scored as 0, normal; 2, loose stools; and 4, watery diarrhea. The disease activity index was defined as the average score for these three parameters. Chronic colitis was induced by repeated administration of 2% DSS on Days 0–5, 10–15, and 20–25. Body weight was monitored daily.

TNBS-induced acute colitis. We used BALB/c mice for TNBS-induced colitis (Elson et al., 1996) because C57BL/6J mice are resistant to TNBS. A Vaseline-lubricated, soft catheter was inserted into the rectum of phenobarbitol-anesthetized BALB/c mice and advanced 3 cm from the anus to administer an intrarectal enema of 100 ml of 2.5 mg TNBS (Sigma-Aldrich, St. Louis, MO) in 50% ethanol. After catheter instillation of the enema solution, the mouse was lifted upward by the tail for 60 s. The mice were then monitored daily for survival. Mice were given a sublethal dose of 100 ml of 1.5 mg TNBS as an enema on Day 0 and euthanized on Day 5 to obtain specimens for histological assessment and evaluation of cytokine expression in colonic tissues. Control group mice were treated with 100 ml of 50% ethanol alone.

Antibiotic administration. To assess the effect of the dose of minocycline on DSS-induced acute colitis, 5, 15, or 30 mg/kg/day of minocycline (Sigma-Aldrich) was administered by intraperitoneal (ip) injection. An ip dose of 30 mg/kg/day minocycline was used in subsequent experiments. Prophylactic treatment was defined as the administration of minocycline before the mice manifested DSS-induced colitis symptoms. Therapeutic treatment was defined as the administration of minocycline after the mice had manifested DSS-induced colitis symptoms. Thus, in the prophylactic treatment protocol, minocycline was administered to the mice from the day on which DSS was first administered and, in the therapeutic treatment protocol, minocycline was administered on the third day after DSS administration. Control mice were given ip injections of phosphate buffered saline (PBS) vehicle. For DSS-induced chronic colitis, minocycline treatment was begun at the same time as DSS was administered. The controls for the treatment of DSS-induced colitis with minocycline were given an antibiotic (60 mg/kg/day metronidazole or ampicillin; Sigma-Aldrich) and subjected to the protocols and schedules described for the minocycline treatment group. To treat TNBS-induced colitis, mice were given minocycline for five consecutive days beginning on the day before the TNBS enemas were administered.

Assessment of colitis. To assess DSS-induced acute colitis, colon tissue from the ileocecal junction to the anal verge was removed on Day 8 after DSS administration. After measuring the length of the colon, the tissue was cut longitudinally and washed with phosphate buffered saline. The distal third of the colon was dissected, fixed with formaldehyde, embedded in paraffin, stained with hematoxylin and eosin (H and E), and subjected to blinded histological assessment. The severity of colitis was assessed using histological scoring as described previously (Williams et al., 2001).

Assay of colonic myeloperoxidase activity. Myeloperoxidase activity was measured as previously described (Bradley et al., 1982) to assess neutrophil infiltration. Fifty milligrams of the distal third of the colon was homogenized in 50 mM phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethyl-ammonium bromide (Sigma-Aldrich). The samples were frozen and thawed three times, and centrifuged at 30,000×*g* for 20 min at 4 °C. The supernatants were diluted 1:30 with

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