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The intestinal anti-inflammatory effect of minocycline in experimental colitis involves both its immunomodulatory and antimicrobial properties

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ABSTRACT

Some antibiotics, including minocycline, have recently been reported to display immunomodulatory properties in addition to their antimicrobial activity. The use of a compound with both immunomodulatory and antibacterial properties could be very interesting in the treatment of inflammatory bowel disease (IBD), so the aim of our study was to evaluate the anti-inflammatory effect of minocycline in several experimental models of IBD. Firstly, the immunomodulatory activity of the antibiotic was tested in vitro using Caco-2 intestinal epithelial cells and RAW 264.7 macrophages; minocycline was able to inhibit IL-8 and nitrite production, respectively. In vivo studies were performed in trinitrobenzenesulfonic acid (TNBS)-induced rat colitis and dextran sodium sulfate (DSS)-induced mouse colitis. The results revealed that minocycline exerted an intestinal anti-inflammatory effect when administered as a curative treatment in the TNBS model, modulating both immune and microbiological parameters, being confirmed in the DSS model; whereas none of the other antibiotics tested (tetracycline and metronidazole) showed anti-inflammatory effect. However, minocycline administration before the colitis induction was not able to prevent the development of the intestinal inflammation, thus showing that only its antimicrobial activity is not enough for the anti-inflammatory effect. In conclusion, minocycline displays an anti-inflammatory effect on different models of rodent colitis which could be attributed to the association of its antibacterial and immunomodulatory properties.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract that comprises two major conditions: Crohn's disease and ulcerative colitis. Although the pathogenesis of IBD remains elusive, the altered and chronic activation of the immune and inflammatory cascade that occurs in genetically susceptible individuals against unknown environmental stimulus may play a key role. There is increasing experimental data that supports a role for luminal bacteria in the initiation and progression of these intestinal conditions; probably related to an imbalance in the intestinal microflora, with a relative predominance of aggressive bacteria and an insufficient amount of protective species [1,2].

For this reason, the manipulation of enteric flora through the administration of antibiotics has been shown to be an important approach in controlling the disease. In fact, it has been reported that remission may be achieved after treatment with antibiotics in intestinal inflammation [3–5]. More recently, remission has been described to be induced in active ulcerative colitis, either by a triple antibiotic therapy [6] or by a synergistic association of antibiotics and corticosteroids [7]. Therefore, the use of

Abbreviations: CINC-1, cytokine-induced neutrophil chemoattractant-1; COX-2, cyclooxygenase-2; DAI, disease activity index; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule-1; IL-, interleukin; iNOS, inducible nitric oxide synthase; LPS, lipopolyssacharide; MCP-1, monocyte chemotactic protein-1; MPO, myeloperoxidase; TFF-3, trefoil factor-3; TNBS, trinitrobenzenesulfonic acid; TNFα, tumor necrosis factor α.

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antibiotics seems to be a rational strategy in the treatment of IBD.

Classically, the beneficial effect exerted by the antibiotics in the treatment of IBD has been mainly attributed to their antimicrobial properties [8–10]. More recently, different studies have reported the ability of many of them to modulate both the innate and the adaptative immune responses by acting directly on different inflammatory cells [11–14]. In particular, minocycline, a semi-synthetic second-generation tetracycline, has been shown to possess anti-apoptotic, immunosuppressive and anti-inflammatory properties in several pathological conditions such as acne vulgaris, periodontitis, rheumatoid arthritis, asthma, scleroderma, neural ischemic damage, Parkinson's disease, spinal cord injury and Huntington disease [15–22]. Furthermore, it has been described to increase the mRNA expression of IL-10 and to reduce TNF-α, inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 expressions in different cell types as well as in animal models [19,20,23].

Therefore, the use of a single compound with both immunomodulatory and antimicrobial activities could be very interesting in the pharmacological treatment of IBD, similarly to what has been shown to occur after antibiotic treatment of systemic and respiratory infections [24,25]. In fact, the administration of intraperitoneal minocycline has been reported to exert intestinal anti-inflammatory effect in two models of experimental colitis in mice [26].

The aim of the present study was to test the intestinal anti-inflammatory effect of orally administered minocycline in different models of colitis. We also aimed to study the role of its immunomodulatory properties and its antibiotic activity on the global beneficial effect achieved. In vitro studies were performed to evaluate the direct immunomodulatory properties of minocycline, both in intestinal epithelial cells (Caco-2 cells) and macrophages (RAW264.7 cells), two cell types actively involved in the intestinal immune response. Afterwards, orally administered minocycline was assayed as prophylactic or curative treatment in the trinitrobenzenesulfonic acid (TNBS) colitis model in rats, and its curative effects were corroborated in the dextran sodium sulfate (DSS) model in mice. The results show that minocycline has an anti-inflammatory effect in several models of rodent colitis that could be attributed to the association of its immunomodulatory properties together with its ability to modulate the intestinal microbiota. Therefore, minocycline points out as a new therapeutic approach for IBD treatment.

2. Methods

All studies were carried out in accordance with the 'Guide for the Care and Use of Laboratory Animals' as promulgated by the National Institute of Health.

2.1. In vitro studies

Caco-2 cells (human colon adenocarcinoma cells) and RAW 264.7 cells (mouse macrophages) were obtained from the Cell Culture Unit of the University of Granada (Granada, Spain) and cultured in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% FBS and 2 mM L-glutamine, in a humidified 5% CO_2 atmosphere at 37 °C. Caco-2 cells were seeded onto 24-well plates at a density of 5×10^5 cells per well and grown until formation of a monolayer. Then, they were pre-incubated for 24 h with different concentrations of each antibiotic (minocycline, tetracycline or metronidazole) ranging from 2 to 100 μM. Afterwards, cells were stimulated with IL-1β (1 ng/ml) for 20 h. Untreated unstimulated cells and untreated cells were used as negative and positive controls. Then the supernatants were collected, centrifuged at $10{,}000 \times g$ for 5 min and stored at −80 °C until IL-8 determination by ELISA (Biosource, Invitrogen™) was performed. RAW 264.7 cells were seeded onto 24-well plates at a density of 5×10^5 cells per well and grown until confluence. They were cultured for 1 h with each of the antibiotics described above and then stimulated with LPS (100 ng/ml); similarly, positive and negative controls were also included. After 20 h, supernatants were collected and centrifuged at $10{,}000 \times g$ for 5 min, and nitrite levels were measured using the Griess assay [27]. Cell viability was examined by the MTT-test described elsewhere [28].

2.2. Trinitrobenzene sulfonic acid (TNBS) model of rat colitis

Female Wistar rats (180–200 g) obtained from the Laboratory Animal Service of the University of Granada (Granada, Spain) were housed in makrolon cages, maintained in an air-conditioned atmosphere with a 12 h light–dark cycle, and they were provided with free access to tap water and food. They were randomly assigned to 6 groups ($n = 10$). Four of them received antibiotic treatment: minocycline (20 or 40 mg/kg), tetracycline (80 mg/kg) or metronidazole (40 mg/kg). The antibiotics were dissolved in 2 ml of distilled water and administered daily by oral gavage. Tetracycline and metronidazole were included as a comparison controls for minocycline: tetracycline and minocycline belong to the same family of antibiotics, sharing chemical structure and antimicrobial spectrum [29]; and metronidazole is an antibiotic which has been widely used in IBD therapy [30]. An untreated TNBS control group and a non-colitic group were also included for reference.

Colonic inflammation was induced in control and treated groups as previously described [31], by the administration of 10 mg of TNBS dissolved in 0.25 ml of 50% ethanol (v/v) by means of a Teflon cannula inserted 8 cm through the anus. Two different treatment protocols were performed: preventive and curative. In the preventive protocol, the antibiotic administration was started 1 week before TNBS instillation and continued up to the day before of the sacrifice of the rats, which took place 2 days after the colitis induction. In the curative protocol, the antibiotic was administered from the day of the colitis induction until the day before of the sacrifice of the rats, 7 days after the induction of the colonic damage. All the rats were sacrificed with an overdose of halothane.

Animal body weights, occurrence of diarrhoea and water and food intake were recorded daily throughout all the experiments. Once the animals were sacrificed, the colon was removed aseptically and placed on an ice-cold plate, longitudinally opened and luminal contents were collected for the microbiological studies. Afterwards, the colonic segment was weighed and its length measured under a constant load (2 g). Each colon was scored for macroscopically visible damage on a 0–10 scale by two observers unaware of the treatment, according to the criteria described by Bell et al. [32].

Representative whole gut specimens were taken from a region of the inflamed colon corresponding to the adjacent segment to the gross macroscopic damage and were fixed in 4% buffered formaldehyde for the histological studies. Equivalent colonic segments were also obtained from the non-colitic group. The remaining colon samples were subsequently sectioned in different longitudinal fragments to be used for biochemical determinations or for RNA isolation.

2.3. Dextran sodium sulfate (DSS) model of mouse colitis

Female C57BL/6J mice (7–9 weeks old; approximately 20 g) obtained from Harlan (Barcelona, Spain) were randomly assigned to two different groups: non-colitic ($n = 10$) and DSS colitic groups ($n = 20$). The colitis was induced by adding DSS (36–50 kDa, MP