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Fumigaclavine C, an fungal metabolite, improves experimental colitis in mice via downregulating Th1 cytokine production and matrix metalloproteinase activity

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Abstract

In the present paper, the effect of Fumigaclavine C, a fungal metabolite, on experimental colitis was examined. Fumigaclavine C, when administered intraperitoneally once a day, significantly reduced the weight loss and mortality rate of mice with experimental colitis induced by intrarectally injection of 2, 4, 6-trinitrobenzene sulfonic acid (TNBS). This compound also markedly alleviated the macroscopic and microscopic appearances of colitis. Furthermore, Fumigaclavine C, given both in vivo and in vitro, showed a marked inhibition on the expression of several inflammatory cytokines, including IL-1β, IL-2, IL-12α, IFN-γ, TNF-α as well as MMP-9 in sacral lymph node cells, colonic patch lymphocytes and colitis tissues from the TNBS colitis mice. Meanwhile, the compound caused a dose-dependent reduction in IL-2 and IFN-γ from the lymphocytes at the protein level and MMP-9 activity. These results suggest that Fumigaclavine C may alleviate experimental colitis mainly via down-regulating the production of Th1 cytokines and the activity of matrix metalloproteinase.

Keywords: Fumigaclavine C; Experimental colitis; TNBS; Cytokines; Matrix metalloproteinase

1. Introduction

Crohn's disease is a complex multifactorial disorder characterized by cytokine-driven and T lymphocyte-dependent inflammation of the intestinal mucosa. It is a chronic, relapsing, and remitting condition of disease and has a high incidence of at least 6/100,000 in western countries [1–3]. Although the etiology of the disease is unknown, it has been suggested that the activation of the mucosal immune system in response to bacterial antigens with consecutive pathologic cytokine production and matrix metalloproteinases activation plays a key pathogenic role [4–8]. Moreover, the mucosa of patients with established Crohn's disease is dominated by $CD4^+$ lymphocytes with a

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type 1 helper-T-cell (Th1) phenotype, which is distinguished by its capacity of producing interferon-γ (IFN-γ) and interleukin-2 (IL-2) [3,4]. To study this disease in mice, a hapten-induced model of colonic inflammation has been introduced by intrarectally delivering 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) and characterized by transmural inflammation associated with weight loss and histopathologic features that mimic some clinical demonstrations of Crohn's disease [9,10].

On the other hand, Fumigaclavine C, an alkaloidal metabolite, was isolated from the culture of *Cephalosporium* sp. IFB-018, an endophytic fungus from the rhizoma of a salinity-tolerant medicinal plant *Imperata cylindrical* by a column chromatography fraction from the chloroform–methanol (1 : 1) extract and was identified by a combination of spectroscopic methods as reported previously by us [11,12]. Although this compound has been discovered as early as 1977 [13], its biological activity is seldom reported. Our previous study has reported its immunosuppressive activity against Con A-induced hepatitis in mice by the mechanisms of inhibiting T cell proliferation, adhesion and TNF-α production [14], which suggests that this compound may have a characteristic to inhibit the T-cell mediated immune response. To further understand the role of Fumigaclavine C in T lymphocyte-directed inflammation, in the present study, we used TNBS-induced colitis model to examine whether it could improve this Th1 type disease.

2. Materials and methods

2.1. Drugs and reagents

The following drugs and reagents were used: Fumigaclavine C (more than 90% of purity, isolated and identified as reported); Cyclosporine A (CsA, Sandoz Ltd, Basel, Switzerland); 2, 4, 6-trinitrobenzene sulfonic acid (TNBS, Sigma, USA); TriPure Isolation Reagent (Roche, USA); M-MLV Reverse Transcriptase (Promega); ELISA kit for murine IL-2 and IFN-γ (Jingmei Biotech Co. Ltd, Shenzhen, China); acrylamide and bis-acrylamide (Shanghai Sangon Biotechnical Ltd. Co., Shanghai, China); collagenase IV, gelatin and Coomassie brilliant blue R-250 (Sigma, USA).

2.2. Animals

Specific-pathogen-free female BALB/c mice, 6–8-week-old, were obtained from Laboratory Animal Center of Shanghai (Shanghai, China). They were maintained in plastic cages at 22 ± 2 °C and kept on a 12 h light–dark cycle with free access to pellet food (Jiangsu Cooperation Medical and Pharmaceutical Company, Nanjing, China) and water. Animal welfare and experimental procedures were carried out strictly in accordance with the guide for the care and use of laboratory animals (National Research Council of USA, 1996) and the related ethical regulations of our university. All efforts were made to minimize animals' suffering and to reduce the number of animals used.

2.3. Induction of colitis

The mice were fasted for 20 h before the experiment. A 3.5 F catheter was then carefully inserted into the colon such that the tip was 4 cm proximal to the anus. To induce colitis, 0.5 mg of the hapten TNBS in 50% ethanol (to break the intestinal epithelial barrier) was slowly injected into the lumen of the colon via the catheter fitted onto a 1-ml syringe. In control experiments, mice received 50% ethanol alone. The total injection volume was 100 μl in both groups allowing TNBS or ethanol to reach the entire colon, including the caecum and appendix. Animals were then kept in a vertical position for 30 s and returned to cages.

2.4. Assessment of inflammation and grading of histological changes

Body weights and survival rate were recorded daily. Mice were treated daily by intraperineal injections of Fumigaclavine C, cyclosporin A or PBS as a vehicle control from day 3 to day 8. All mice were sacrificed nine days after the TNBS administration. Sacral lymph nodes, colonic patches and colons were harvested on sacrifice. After removal of faecal materials, colons from the mice treated variously were fixed in 10% buffered formalin phosphate. They were then embedded in paraffin, cut into 4-μm-thick serial sections and stained with hemotoxylin and eosin. Histological grading was done according to