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Curcumin, a *Curcuma longa* constituent, acts on MAPK p38 pathway modulating COX-2 and iNOS expression in chronic experimental colitis

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Abstract

Ulcerative colitis (UC) is a nonspecific inflammatory disorder characterized by oxidative and nitrosative stress, leucocyte infiltration and up-regulation of pro-inflammatory cytokines. Mitogen-activated protein kinases (MAPKs), such as the p38 and the c-Jun N-terminal kinase (JNK) modulate the transcription of many genes involved in the inflammatory process. Curcumin is a polyphenol derived from *Curcuma longa*, which is known to have anti-inflammatory activity. The aim of this study was to study the effects and mechanisms of action of curcumin, on chronic colitis in rats. Inflammation response was assessed by histology and myeloperoxidase activity (MPO). We determined the production of Th1 and Th2 cytokines and nitrites in colon mucosa, as well as the expression of inducible nitric oxide synthase (iNOS), cyclo-oxygenase(COX)-1 and-2 by western blotting and inmmunohistochemistry. Finally, we studied the involvement of MAPKs signaling in the protective effect of curcumin in chronic colonic inflammation. Curcumin (50–100 mg/kg/day) were administered by oral gavage 24 h after trinitrobenzensulfonic acid (TNBS) instillation, and daily during 2 weeks before sacrifice. Curcumin significantly attenuated the damage and caused substantial reductions of the rise in MPO activity and tumour necrosis factor alpha (TNF)-α. Also curcumine was able to reduce nitrites colonic levels and induced down-regulation of COX-2 and iNOS expression, and a reduction in the activation of p38 MAPK; however, no changes in the activation of JNK could be observed. In conclusion, we suggest that inhibition of p38 MAPK signaling by curcumin could explain the reduced COX-2 and iNOS immunosignals and the nitrite production in colonic mucosa reducing the development of chronic experimental colitis.
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Keywords: Curcumin; Colitis; Neutrophils; Inducible nitric oxide synthase (iNOS); Cyclo-oxygenase (COX)-2; Mitogen-activated protein kinases (MAPKs)

1. Introduction

Inflammatory bowel disease (IBD), collectively referred to as Crohn's disease (CD) and ulcerative colitis (UC), is a nonspecific inflammatory disorder involving mainly the colonic mucosa and submucosa. Activated immune cells, primarily represented by neutrophils,

Abbreviations: CD, Crohn's disease; COX, Cyclo-oxygenase; CUR, Curcumin; HETAB, Hexadecyl-trimethylammonium bromide; IBD, Inflammatory bowel disease; IFN, Interferon; IL, Interleukin; JNK, c-Jun N-terminal kinase; MAPKs, Mitogen-activated protein kinases; MPO, Myeloperoxidase activity; NF-κB, Nuclear factor kappa B; iNOS, Inducible nitric oxide synthase; NO, Nitric oxide; TMB, 3,3′,5,5′-tetramethylbenzidine; TNBS, Trinitrobenzenesulfonic acid; TNF-α, Tumor necrosis factor alpha; UC, Ulcerative colitis.

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macrophages and cytotoxic T cells, play the aggressor role by attacking and destroying the intestinal barrier either directly through physical contact or indirectly through the release of reactive oxygen and nitrogen metabolites. Reactive oxygen species are now increasingly recognized to be involved in cell growth, signaling and gene expression [1]. Furthermore, reactive oxygen species can activate diverse downstream signaling pathways, such as MAPKs or the nuclear factor NF-kappa B (NF-κB), thus modulating a number of different steps in the inflammatory cascade. These include production of pro-inflammatory cytokines (tumour necrosis factor alpha (TNF-α), interleukin (IL)-1β, interferon gamma (IFN-γ), IL-12, and IL-6) in different cell-types, the expression of receptors essential for neutrophils activation and chemotaxis and certain proteins, important determinants of colonic damage, i.e. cyclo-oxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) [2,3].

COX-2 is expressed as an early response to pro-inflammatory mediators and mitogen stimuli. In our previous studies we have observed that the increased prostaglandins production during chronic colitis is dependent upon the activity of COX-2 [4,5]. Excessive production of nitric oxide (NO) by iNOS in chronic colitis may be detrimental to the integrity of the mucosa based on the generation of reactive nitrogen species which causes cellular degeneration in various tissues, contributing to the development of intestinal damage. iNOS acts in synergy with COX-2 to promote the inflammatory reaction. Furthermore, both COX-2 and iNOS expressions are up-regulated by activated MAPKs in intestinal epithelial cells [6].

Curcumin is a polyphenol found in the dietary spice, extracted from dried rhizomes of the perennial herb turmeric (*Curcuma longa* Linn), a member of the ginger family. Curcumin is used as a spice to give the specific flavour and yellow colour to curry [7]. As a traditional medicine, turmeric has also been widely used for centuries to treat inflammatory disorders in its original countries such as arthritis, colitis and hepatitis [8]. There are recent reports which document that curcumin decreases the degree of inflammation associated with experimental colitis [9–12]. Based upon these data there is no doubt that the polyphenol plays an important role in anti-inflammatory responses in colon. However, there are many interesting questions regarding the therapeutic activity of curcumin in IBD. Thus, the aim of this study was to get a better understanding of the effects and mechanisms of action of curcumin, on the chronic injury caused by intracolonic administration of trinitrobenzensulfonic acid (TNBS) in the rat. Inflammatory response was assessed by histology and myeloperoxidase activity (MPO) was measured as an index of neutrophil infiltration in the mucosa. Th1 and Th2 cytokines production such as TNF-α and IL-10 were also carried out. We determined the production of NO in colonic mucosa as well as, the expression of iNOS, COX-1 and-2 by western blotting and immunohistochemistry. Finally, we studied the involvement of p38 MAPK and JNK signaling pathways in the protective effect of curcumin in chronic colonic inflammation.

2. Material and methods

2.1. Experimental animals

Male and female Wistar rats supplied by the Animal Services, Faculty of Medicine, University of Seville, Spain, and weighing 180–220 g, were placed singly in cages with wire-mesh floors at a controlled room temperature 24–25 °C, humidity 70–75%, lighting regimen of 12L/12D and were fed a normal laboratory diet (Panlab, Barcelona, Spain). Rats were deprived of food for 24 h prior to the induction of colitis, but were allowed free access to tap water throughout. They were randomly assigned to groups of 8–14 animals. The experiments followed a protocol approved by the local animal Ethics Committee and the Local Government. All experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Counsel 86/609/EC).

2.2. Induction of colitis

Colitis was induced according to the procedure described by Morris et al. [13]. Briefly, rats were lightly anesthetized with penthobarbital following a 24 h fast, and then a medical-grade polyurethane cannula for enteral feeding (external diameter 2 mm) was inserted into the anus and the tip was advanced to 8 cm proximal to the anus verge. TNBS (Sigma Aldrich-Company Ltd., Spain) dissolved in 50% (v/v) ethanol was instilled into the colon through the cannula (30 mg in a volume of 0.25 ml) to induce chronic colitis.

Following the instillation of the hapten, the animals were maintained in a head-down position for a few minutes to prevent leakage of the intracolonic instillate. Control groups were separated for comparison with TNBS/ethanol instillation: rats in the sham group received physiological saline, instead of TNBS solution. Curcumin (50–100 mg/kg; Sigma-Aldrich, Company Ltd. Spain) was emulsified in 0.9% saline solution and Tween 20%, and administered by oral route 24 h after TNBS instillation and daily during the 2 weeks before the sacrifice of the rats. The control group also received the vehicle solution by oral route. The animals were sacrificed, using an overdose of anaesthetic. The rats were checked daily for behaviour, body weight, and stool consistency.

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