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Study of curcumin immunomodulatory effects on reactive astrocyte cell function

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ABSTRACT

Multiple sclerosis (MS) is considered an inflammatory and neurodegenerative disease of the central nervous system (CNS) which most often presents as relapsing–remitting episodes. Recent evidence suggests that activated astrocytes play a dual functional role in CNS inflammatory disorders such as MS. In this study, we tried to induce anti-inflammatory functions of astrocytes by curcumin. The effects of curcumin were examined on human a astrocyte cell line (U373-MG) induced by lipopolysaccharide (LPS) in vitro. Matrix metalloproteinase (MMP)-9 activity was assessed by gelatin zymography. Cytokine levels were evaluated by quantitative ELISA method and mRNA expression was measured by real-time PCR. We found that curcumin decreased the release of IL-6 and reduced MMP-9 enzyme activity. It down-regulated MCP-1 mRNA expression too. However, curcumin did not have significant effects on the expression of neurotrophin (NT)-3 and insulin-like growth factor (IGF)-1 mRNAs. Results suggest that curcumin might beneficially affect astrocyte population in CNS neuroinflammatory environment lean to anti-inflammatory response and help to components in respects of CNS repair. Our findings offer curcumin as a new therapeutic agent with the potential of regulating astrocyte-mediated inflammatory diseases in the CNS.

1. Introduction

Multiple sclerosis (MS) is a chronic disorder of the central nervous system (CNS) characterized by inflammation, demyelination and axonal degeneration which most often present as relapsing–remitting episodes and the global estimated number of people with MS has increased from 2.1 million in 2008 to probably higher than 2.5 million in 2014. The exact mechanisms of MS etiology remains unknown, however recent findings suggest that there are interactions between environmental factors and gene mutations as MS triggers [1]. Diverse pathophysiological characteristics appear during the different episodes of disease, such as breakdown of the blood–brain barrier (BBB), autoimmune spells, and axonal and myelin sheath damages [2]. The activation of autoreactive CD4+ T cells in the periphery is considered the first important immunological event in MS. This is confirmed by data obtained from the animal model experimental autoimmune encephalomyelitis (EAE), which is initiated by systemic immunization with neuronal autoantigens or by transfer of CD4+ T cells sensitized with neuronal antigens [3]. Further studies on EAE revealed that pro-inflammatory Th17 cells are also critically engaged in the onset of disease pathogenesis [4]. Given the prominence of inflammatory changes in acute MS lesions, therapy for the disease has focused particularly on anti-inflammatory strategies [5]. Several approved therapies are now available, albeit with only moderate impact on disease course [6]

The curcumin, a hydrophobic polyphenol, is a principal active constituent of turmeric (*Curcuma longa*). The yellow color of the turmeric is due to the curcumin compound. In traditional Indian medicine, curcumin has been used to cure inflammation [7]. The curcumin has currently received much consideration for its anti-inflammatory, antioxidant, and antitumor activities [8]. Curcumin has a lipophilic feature, can pass through all cell membranes, and thus applies its intracellular effects

Abbreviations: APC, antigen presenting cell; BBB, brain blood barrier; CD, cluster of differentiation; CNS, central nervous system; CTLA, cytotoxic T lymphocyte antigen; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; IGF, insulin-like growth factor; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MCP, monocyte chemotactic protein; MMP, matrix metalloproteinase; MS, multiple sclerosis; NF-κB, nuclear factor-kappa B; NO, nitric oxide; NT, neurotrophin; OPC, oligodendrocyte progenitor cell; PenStrep, penicillin/streptomycin; PCR, polymerase chain reaction; STAT, signal transduction and activator of transcription; RT, reverse transcription; TGF, transforming growth factor; TNF, tumor necrosis factor.

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[9]. Curcumin has restrictive pharmaceutical role because of its extremely low aqueous solubility, rapid systemic removal, insufficient tissue absorption and degradation at alkaline pH, which notably decrease its bioavailability [10]. The anti-inflammatory actions of curcumin seem to be closely related to the suppression of proinflammatory cytokines and release of mediators [11]. Curcumin also inhibited tumor necrosis factor (TNF)-α and nitric oxide (NO) release in a dose-dependent manner by inhibiting differentiation and proliferation of Th17 cells and down-regulating NF-κB activation [12]. It inhibited proinflammatory cytokine interleukin (IL)-23, IL-1β, IL-6, and transforming growth factor (TGF)-β release in lipopolysaccharide (LPS)-stimulated mature dendritic cells by inhibiting NF-κB activation with MAPK signal pathway suppression [13]. In addition, curcumin showed inhibitory effect on IL-12-induced STAT4 phosphorylation in human T cells [14]. Although direct suppressive effects of curcumin on superantigen induced proliferation of T cells were demonstrated in several studies, some studies also demonstrated that T cells fail to get proper amount of co-stimulatory signals from curcumin-treated antigen presenting cells (APCs), as curcumin (20–30 μM) aborted the upregulation of CD86 and CD83 in response to the APC maturation stimuli [15]. Through its various anti-inflammatory effects, it may have a role in the treatment of MS [9]. In a study about the impact of curcumin on formation and aggregation of amyloid-β in in vivo and in vitro models of Alzheimer disease, researchers demonstrated that the low molecular weight and polar structure of curcumin allowed it to penetrate the BBB and bind to the beta-amyloids preventing them from forming the disease-causing plaque [16]. In contrast to nonsteroidal anti-inflammatory drugs whose adverse side effects include gastrointestinal ulceration and liver or kidney toxicity, curcumin seems to be relatively non-toxic, even in clinical trials for prevention of relapse of ulcerative colitis [17].

Astrocytes, the most numerous brain glial cell population, have a fundamental role in the maintenance of homeostasis within the CNS, even though they are also closely associated with both neuroprotection and neurodegradation when they are activated in response to stimuli and disease states [18]. These cells are also involved in the formation and support of the BBB and are essential for neuronal functions [19]. Astrocytes produce neurotrophin-3 (NT-3) and insulin-like growth factor-1 (IGF-1) to support oligodendrocyte progenitor cell (OPC) survival and promote neurogenesis. Upon pathological conditions, activated astrocytes increase production of these factors [20,21]. IL-6, TNF-α, and IL1-β are released by astrocytes during inflammation and increase BBB permeability by acting particularly on endothelial cells and tight junctions [22]. By expression of chemokines like the monocyte chemotactic protein (MCP)-1/(CCL-2), astrocytes may have significant effects on migration of immune cells across the BBB due to the proximity of astrocytic end feet to blood vessels [23]. Other studies have shown that human astrocytes, during inflammation, inhibit antigen-specific T cell proliferation through upregulation of cytotoxic T lymphocyte antigen (CTLA)-4 and expression of anti-inflammatory cytokines including IL-10 and TGF-β [24,25]. Astrocytes are the major sources of matrix metalloproteinases (MMPs) as well as the extracellular matrix components. MMP activities have been implicated in many of the neuro-physiological and neuro-pathological situations [26].

According to the dual role of astrocytes in triggering and inhibiting the inflammation in CNS by pro and anti-inflammatory cytokine release [27] and also the confirmed effects of curcumin on T cell regulation in inflammatory responses [28], in this study, we investigated the effects of curcumin on astrocytic U373 cell line functions by using a LPS-induced inflammatory in vitro model.

2. Material & methods

2.1. Reagents and cell line

Curcumin and LPS were purchased from Sigma-Aldrich (St. Louis, US). Cell culture reagents and saline buffers were purchased from Gibco (Life Technologies, US). Penicillin/streptomycin (PenStrep) antibiotic solution was obtained from EuroClone, Italy. All chemicals including electrophoresis-grade gelatin (type b) were purchased from Sigma-Aldrich. The human astrocytic U-373 MG cell line was obtained from the National Cell Bank of Iran (NCBI, Pasteur Institute).

2.2. Cell culture

The U373-MG cells were maintained in high glucose Dulbecco's modified Eagle medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1× PenStrep antibiotic solution. Cells were kept by incubation at 37 °C in a humidified atmosphere of 5% CO_2. When cells reached approximately 80–90% of confluency, they were detached by trypsinization with 0.25% Trypsin, and 0.1 mM EDTA for 2 min. Cells were pelleted by centrifugation at 1250 RPM for 6 min, then re-suspended in fresh medium (to the dilution required) and transferred to clean culture 25 cm^2 flasks. In order to determine the viability, cells were counted using the trypan blue exclusion technique. Finally, cells were allowed to attach to the culture plate and stabilize overnight at 37 °C before treatment.

2.3. Cytotoxicity assay (MTT)

Cytotoxicity of LPS-induced astrocytes treated with curcumin were detected using MTT (3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide) assay. Briefly, triplicate samples of 3×10^3 cells were plated in 96-well flat-bottomed plates in DMEM, and incubated at 37 °C overnight. Then, cells were stimulated with 0.5 μg/ml LPS. After 30 min of incubation at 37 °C, cells were treated with 0, 1, 2.5, 5, 10 and 20 μM curcumin over different incubation times (24 and 48 h). At the end of each time, they were exposed to 10 μl of the MTT reagent for 4 h at 37 °C. Reaction was stopped by removing the medium and the dye was dissolved in absolute ethanol. The difference between the absorbance of each sample at 560 nm and 690 nm was measured using an automatic microplate reader. The viability of untreated cells was considered to be 100%.

2.4. Treatment

Cells were seeded in a 24-well plate at a density of 5×10^4 and then exposed to LPS at a concentration of 0.5 μg/ml for 30 min. As assessed by MTT assay, different non-toxic concentrations of curcumin were added (0, 2.5 and 5 μM). After each treatment, the cell culture supernatants were collected and frozen at −70 °C until assayed for cytokine concentration and MMP-9 activity using gelatin zymography. Cells, after washing, were kept at −70 °C for RNA isolation.

2.5. Detection of MMP-9 by zymography

Zymography was done for semiquantitative analysis of MMP9 (gelatinase B) levels secreted into the culture medium as described in our previous study in 2005 [29] with slight modifications. In brief, samples were mixed with 5 μl of 4× SDS sample buffer (2% w/v SDS, 40% urea, 200 mM Tris–borate, pH 8.6, and 0.001% bromophenol blue) in the absence of a reducing agent and resolved by electrophoresis at 80 V for 3 h on 7% polyacrylamide gel containing 1% SDS and gelatin at a final concentration of 0.5 mg/ml. Thereafter, gels were washed twice in 2.5% Triton X-100 for 30 min each to remove the SDS and then were incubated for 18 h at 37 °C in reaction buffer (0.1 M Tris–HCl, 10 mM CaCl, and pH 7.4). After staining the gel with 0.2% Coomassie Brilliant Blue R-250, gelatinolytic activity of MMP-9 was visualized as a clear band in the uniformly stained background. The optical density (OD) of the area of each clear band was determined using the ImageJ software (NIH, US).