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The relationship between the anti-inflammatory effects of curcumin and cellular glutathione content in myelomonocytic cells

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Abstract

Oxidative stress plays an important role during inflammatory diseases and recent therapies have focused on antioxidant administration to diminish oxidative stress and to arrest inflammatory processes. In this study, we investigated the impact of the GSH modulating effects of curcumin, a naturally derived polyphenol, on inflammatory processes in myelomonocytic U937 cells.

One hour after administration of 10 μmol/l curcumin reactive oxygen species (ROS) production was significantly increased in undifferentiated U937 cells (+43%). Twenty-four hour after addition of curcumin, a significantly decreased ROS concentration was found (−32%), whereas GSH (+110%) and GSSG (+88%) content increased. A higher concentration of curcumin (25 μmol/l) caused an even stronger increase of GSH (+145%) and GSSG (+101%), but significantly decreased percentage of living cells to 84%.

The increased GSH content of differentiated U937 cells after pre-incubation with curcumin was associated with lowered ROS production, nuclear factor kappa B (NFκB) activation (−34%) and tumor necrosis factor alpha (TNF-α) secretion (−51%) after LPS exposure. Curcumin inhibited TNF-α formation was also seen after GSH depletion by buthionine sulfoximine (BSO).

This study shows that the antioxidative effects of curcumin are preceded by an oxidative stimulus, which is time and dose-dependent. Excessive concentrations of curcumin may even harm cells, as cell viability was decreased, in spite of elevated GSH contents. There was no clear relationship between intracellular GSH concentrations and the anti-inflammatory effects of curcumin.

Keywords: Curcumin; Antioxidative; Prooxidative; Anti-inflammatory; Glutathione; Reactive oxygen species

1. Introduction

There is increasing evidence that oxidative stress plays a crucial role during development and progression of inflammatory diseases. The most important intracellular antioxidative defense against oxidative stress is the tripeptide glutathione (GSH), which is oxidized to glutathione disulfide (GSSG) while it scavenges free radicals. During inflammatory diseases reduced levels of GSH and/or increased levels of free radicals are detected [1,2], indicating the involvement of oxidative stress. Recent publications have shown that an oxidized cytosolic environment amplifies activation of nuclear factor kappa B (NFκB), which plays a critical role during inflammatory processes by activating many genes encoding for proinflammatory cytokines and immunoregulatory mediators [3]. Some publications have also suggested that reactive oxygen species (ROS) influence inflammatory processes through mechanisms that are dependent on NFκB [4]. Rose et al. have shown that the production of tumor necrosis factor alpha (TNF-α) is regulated by a ROS-activated NFκB pathway in Kupffer cells [5]. Additionally, pre-treatment of human bronchial epithelial cells with *N*-acetyl cysteine, a well known antioxidant, decreases TNF-α-induced activation of NFκB and IL-8 promoter-mediated reporter gene expression [6]. Moreover, the impact of GSH on immune function has been investigated and it has been discovered that GSH depletion in rats impairs T-cell and macrophage

Abbreviations: BSO, buthionine sulfoximine; DMSO, dimethyl sulfoxide; EMSA, electrophoretic mobility shift assay; GSH, glutathione; GSHt, total glutathione; GSSG, glutathione disulfide; LPS, lipopolysaccharide; MCF, mean channel fluorescence; NFκB, nuclear factor kappa B; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha

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immune function [7]. In this respect, recent investigations for treatment of inflammatory diseases have focused on antioxidant administration to diminish oxidative stress and thus arrest inflammatory processes. Therefore, discovery and investigation of new substances with antioxidative capacities are of particular clinical interest.

Curcumin (diferuloylmethane) is a low molecular weight polyphenol and the main component of the rhizomes of the plant *Curcuma longa* L. Most experimental studies of this substance focus on its cancer chemopreventive effects and it has been demonstrated that curcumin inhibits tumor initiation and promotion in various tissues during animal studies [8,9]. In this respect, some clinical studies have been published recently [10,11]. Several other experimental studies have revealed that curcumin is a potent antioxidant, as it is able to scavenge ROS [12,13], increase GSH content [14,15] and decrease lipid peroxidation [16]. These antioxidative effects of curcumin might be useful for the prevention or treatment of inflammatory processes.

This study was therefore designed to investigate the impact of curcumin-evoked increase in GSH on the occurrence of ROS and inflammatory reactions in myelomonocytic cells. Interestingly, our results revealed that at least TNF-α formation was not dependent on cellular GSH content.

2. Materials and methods

2.1. Chemicals

All chemicals were derived from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated and were of the highest grade obtainable. Curcumin was dissolved in DMSO so that the final concentration of DMSO in the cell culture medium never exceeded 0.1%. All substance stock solutions were prepared freshly before use.

2.2. Cell culture

The human myelomonocytic cell line U937 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). Cells were maintained in continuous cell suspension at 37 °C under 5% CO_2 and humidified air in RPMI 1640 medium (BioWhittaker, Verviers, Belgium) supplemented with 2 mmol/l glutamine (Gibco, Paisley, UK) and 10% fetal bovine serum (FBS; Linaris, Bettingen, Germany).

2.3. Treatment of undifferentiated U937 cells

Undifferentiated U937 cells were used to study the antioxidative effects of curcumin. In former studies we found that GSH content of U937 cells reached a maximum after 24 h cultivation at standard conditions [17]. Therefore, 0.2×10^6 cells/ml were incubated in RPMI medium containing curcumin (1–25 μmol/l) for 24 h at standard conditions. Respective controls were treated with an equal volume of DMSO. After incubation cell counts of living and dead cells were examined by trypan blue staining and cell viability was calculated. GSH and GSSG levels of the cells were assessed after 24 h incubation with increasing concentrations of curcumin (1–25 μmol/l). The production of ROS was measured 1, 3, 6, 12 and 24 h after addition of 10 or 25 μmol/l curcumin.

2.4. Differentiation of U937 cells with PMA

As undifferentiated U937 cells fail to produce TNF-α upon lipopolysaccharide (LPS) stimulation (data not shown), we used a 24 h incubation period with 25 ng/ml phorbol 12-myristate 13-acetate (PMA) to differentiate cells and sensitize them to LPS, according to Refs. [18,19]. To investigate influence of PMA on differentiation of the cells, expression of surface antigens (CD11b, CD14) was quantified by flow cytometry using a direct immunofluorescence staining technique. Nondifferentiated (=control) and PMA-differentiated U937 cells were collected with HBSS containing 0.1% sodium azide and 1% FBS, washed and counted. Aliquots (20 μl) of antibodies (CD11b-FITC and CD14-FITC, Immunotech, Marseille, France) were added to stain the cells. After 30 min of incubation at 4 °C, cells were washed twice, resuspended in 500 μl HBSS and analyzed by flow cytometry (XL-EPICS, Beckman Coulter, Fullerton, CA).

2.5. Treatment of PMA-differentiated U937 cells

After 24 h incubation with PMA, cells were pelleted by centrifugation and PMA-containing medium was removed. To investigate the influence of GSH modulation on inflammatory processes fresh medium containing 10 μmol/l curcumin, 150 μmol/l buthionine sulfoximine (BSO), an inhibitor of GSH synthesis, or curcumin (10 μmol/l) + BSO (150 μmol/l) was added and cells were again incubated for 24 h. After that, on one hand GSH and GSSG levels were measured. On the other hand 1 μg/ml LPS (serotype: O111:B4) [18] was added to pre-treated cells. ROS formation was assessed before as well as 3 and 6 h after addition of LPS. Activation of NFκB was examined 30 min after LPS addition, as former experiments revealed the strongest activation at this time point (data not shown). TNF-α production was assessed 6 h after LPS administration.

2.6. Determination of GSH and GSSG

Levels of GSH and GSSG were determined using the Calbiochem® GSH/GSSG Ratio Assay Kit (Calbiochem, San Diego, CA), based on the method originally described by Tietze [20]. Briefly, $(5–10) \times 10^6$ cells were harvested