Biochemistry and Biophysics Reports xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Crocin, a plant-derived carotenoid, modulates microglial reactivity

Mücella Arikan Yorgun, Khalid Rashid, Alexander Aslanidis, Charlotte Bresgen, Katharina Dannhausen, Thomas Langmann*

Laboratory for Experimental Immunology of the Eye, Department of Ophthalmology, University of Cologne, D-50931 Cologne, Germany

ARTICLE INFO

Keywords:
Crocin
Microglia
Retina

ABSTRACT

Microglia activation plays an important role in immune responses in the CNS including the retina. Crocin, a plant-derived carotenoid, has been reported to possess anti-inflammatory, anti-apoptotic and anti-oxidative capacity in models of retinal damage and degeneration. If these neuroprotective effects could be mediated by direct modulation of microglial cells is unclear. Here, we examined the direct effects of crocin on key functions and pro-inflammatory gene expression in lipopolysaccharide (LPS)-activated BV-2 microglia. We found that crocin stimulation strongly promoted filopodia formation and markedly increased microglial phagocytosis, two important parameters relevant for physiological microglia functions. Moreover, crocin significantly reduced gene expression of the pro-inflammatory markers IL6, CCL2, and iNOS in LPS-challenged BV-2 cells and potently blocked NO production in these microglia. The observed immunomodulatory effects of crocin were not mediated by general inhibition of NFkB nuclear translocation. Our findings indicate that many of the anti-inflammatory effects of crocin demonstrated in animal models of neuronal degeneration could be mediated by its direct effects on microglia homeostasis.

1. Introduction

Microglial cells are resident macrophages of the central nervous system including the retina and have important roles in retinal and neuronal homeostasis [1,2]. In the “surveillance” state, they have a small soma with fine cellular processes and ramified morphology, which actively scan their environment [3]. Under pathological conditions microglia transform to “reactive” states, which involves changes in morphology into an amoeboid shape, their migration to injury site and the release of pro-inflammatory and cytotoxic factors [4]. While microglial activation may have a protective role in the maintenance of retinal integrity [5], microglial may even actively contribute to retinal degenerative diseases [6]. Consequently, chronic activation of these cells has been documented in various neurodegenerative diseases of the retina, including age-related macular degeneration [7], inherited photoreceptor dystrophies [8], and glaucoma [9]. Therefore, modulation of microglial reactivity emerged as therapeutic strategy to treat retinal degenerative diseases [10].

Recent years witnessed a growing interest in the discovery of natural compounds that have an impact on neuroinflammatory processes. Among these natural immunomodulators, saffron has been used worldwide in traditional medicine [11]. Saffron and its active ingredients mainly crocin, crocetin and safranal exert anti-proliferative, anti-tumor, anti-inflammatory, anti-oxidant, anti-apoptotic, and hepatoprotective effects [12]. Administration of saffron components in different models of retinal damage and degeneration showed neuroprotective effects [13,14]. Likewise, safranal could slow down photoreceptor degeneration in the P23H rat model of retinitis pigmentosa, reflected by improved a- and b-wave amplitudes in electroretinographic recordings and a preserved vascular network [14]. Similarly, crocin and crocetin had protective effects against light induced photoreceptor degeneration in vitro and in vivo [15–17]. Moreover, crocin significantly prevented retinal ganglion cell apoptosis after retinal ischaemia/reperfusion injury [18]. There are also indications, that saffranal supplementation in patients with age-related macular degeneration (AMD) improved their retinal function as detected by increased focal electroretinograms (fERGs) amplitudes [19]. Despite these widely reported neuroprotective effects of crocin, it is unclear whether these beneficial effects may be mediated, at least partially, by modulation of microglial reactivity. In this study, we aimed to investigate the direct immune-modulatory effect of crocin in BV-2 microglial cells.

* Correspondence to: Laboratory for Experimental Immunology of the Eye, Department of Ophthalmology, University of Cologne, Joseph-Stelzmann Strasse 9, D-50931 Cologne, Germany.
E-mail address: thomas.langmann@uk-koeln.de (T. Langmann).

http://dx.doi.org/10.1016/j.bbrep.2017.09.007
Received 9 June 2017; Received in revised form 11 September 2017; Accepted 24 September 2017

2. Materials and methods

2.1. Cell culture

BV-2 microglia were cultured in RPMI 1640 with 5% fetal calf serum (FCS) supplemented with 2 mM L-glutamine, 1% penicillin/streptomycin and 195 nM β-mercaptoethanol at 37 °C in a humidified atmosphere of 5% CO2 as previously described [20]. BV-2 cells were pre-incubated with 200 μM crocin or PBS as vehicle control in fresh medium, without FCS, for 30 min. Afterwards, the cells were stimulated with 50 ng/ml LPS, 200 μM crocin, or 50 ng/ml LPS + 200 μM crocin for 24 h. These stimulation conditions were adopted from preliminary experiments which revealed that 200 μM crocin was the most effective dose and had no cytotoxic effects (data not shown). 661 W photoreceptor-like cells were a gift from Prof. Muayyad Al-Ubaidi (Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA), and the culture conditions have been described elsewhere [21].

2.2. Phalloidin-TRITC staining

BV-2 microglial cells were seeded on cover slips in six-well plates and cultivated and stimulated as described before. Afterwards cells were fixed with 4% formaldehyde, permeabilized with 0.1% Triton X-100, and F-actin was fluorescently labeled using 0.1 μg/ml Phalloidin-TRITC (Sigma-Aldrich). Nuclei were stained using DAPI, and the cover slips were mounted with fluorescent mounting medium (Dako Cytomation). Photomicrographs were taken with an AxioImager. M2 plus ApoTome2 microscope (Carl Zeiss). Quantitative scoring of microglial ramification was performed as described previously using a grid-cross analysis [6].

2.3. Phagocytosis assay

Phagocytosis assay 661 W photoreceptor cells were starved for two weeks with serum deprivation, harvested and fluorescently labeled using CellTracker CM-DiI (Invitrogen, Carlsbad, CA, USA). BV-2 cells were left to adhere on coverslips overnight and pre-treated with PBS or crocin for 30 min. Afterwards microglial cells were cultivated with or without LPS for further 24 h. In the following 400 μl labeled apoptotic photoreceptor material was added. After a further cultivation period of 6 h, microglial cells were washed and nuclei were stained with DAPI. Fluorescence micrographs were then taken and ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to determine the fluorescent intensities. After subtracting background intensities from both DsRed (phagocytosed apoptotic photoreceptor) and DAPI (total microglia cell number) signals, the Phagocytosis Index was determined by dividing the corrected DsRed signal by the corrected DAPI signal and giving these numbers in percent [20].

2.4. RNA isolation and real-time RT-PCR

Total RNA was extracted from microglia cells according to the manufacturer's instructions using the NucleoSpin® RNA Mini Kit (Macherey-Nagel, Dueren, Germany). RNA was quantified spectrophotometrically using a NanoDrop 2000 (Thermo Scientific) and then stored at −80 °C. First-strand cDNA synthesis was performed with the RevertAid™ H Minus First strand cDNA Synthesis Kit (Fermentas, Schwerte, Germany). Amplifications of 50 ng cDNA were performed with an ABI7900HT machine (Applied Biosystems, Carlsbad, CA, USA) in 10 μl reaction mixtures containing 1 × TaqMan Universal PCR Master Mix (Applied Biosystems), 200 nM of primers and 0.25 μl of dual-labeled probe (Roche ProbeLibrary, Roche Applied Science, Basel, Switzerland). The reaction parameters were as follows: 2 min 50 °C hold, 30 min 60 °C hold and 5 min 95 °C hold, followed by 45 cycles of 20 s 94 °C melt and 1 min 60 °C anneal/extension. Primer sequences and Roche Library Probe numbers were as follows: CCL2, forward primer 5′-catccacgtgttggctca-3′, reverse primer 5′-gatcatcttgctggtgaatgagt-3′, probe #62; IL6, forward primer 5′-gatggatgctaccaaactggat-3′, reverse primer 5′-ccaggtagctatggtactccaga-3′, probe #6; iNOS, forward primer 5′-ctttgccacggacgagac-3′, reverse primer 5′- tcattgtactctgagggctga-3′, probe #13; Measurements were performed in triplicates. ATPase, forward primer 5′-ggcacaatgcaggaaagg-3′, reverse primer 5′-tcagcaggcacatagatagcc-3′, probe #77. ATPase expression was used as reference gene and the results were analyzed with the ABI sequence detector software version 2.4 using the ΔΔCt method for relative quantification [6].

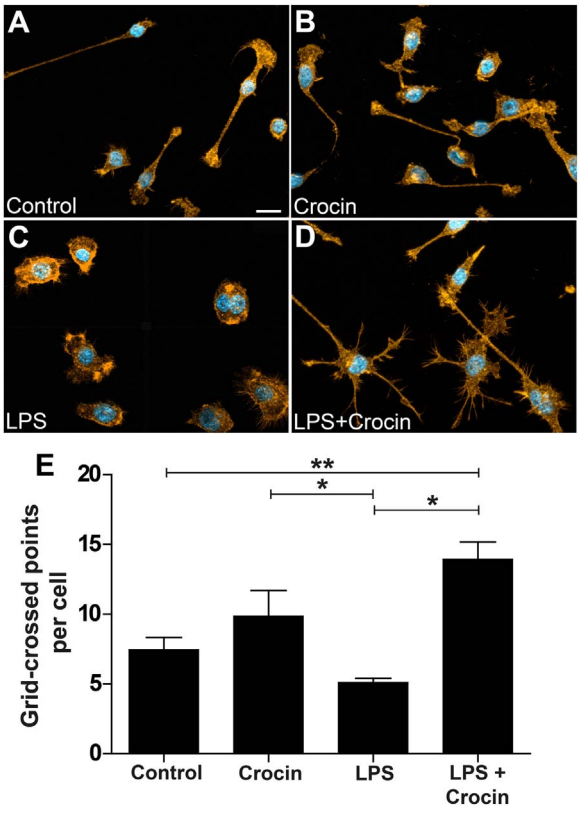

Fig. 1. Crocin promotes microglial filopodia formation. Representative images of Phalloidin-TRITC/DAPI labeled BV-2 microglial cells showing morphological changes in response to stimulation with vehicle (A), 200 μM crocin (B), 50 ng/ml LPS (C), and 50 ng/ml LPS plus 200 μM crocin (D). Cells were pre-treated with 200 μM crocin for 30 min, followed by stimulation with 50 ng/ml LPS for further 24 h. Scale bar = 20 μm. (E) Quantification of microglial ramification was performed using a grid-cross counting method [6]. Data show mean ± SEM (n = 13–18 cells/group in three independent images), **P < 0.01 for LPS + crocin- versus control, *P < 0.05 for LPS + crocin- versus LPS alone, and *P < 0.05 for LPS versus crocin.

2.5. Nitrite assay

The nitrite concentration in culture supernatants was determined as an indicator of nitric oxide (NO) production using the Griess reagent system (Promega). 50 μl cell culture supernatants were incubated with an equal volume of Griess reagent in each well of a translucent 96-well plate. After incubation for 30 min at room temperature, the absorbance was read at 540 nm on an Infinite F200 pro plate reader (Tecan). Nitrite concentrations for each sample were calculated from a sodium nitrite standard curve.

2.6. Immunocytochemistry

BV-2 cells were seeded on sterile cover slips and were cultivated and stimulated as described before. Afterwards cells were fixed with 4%