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Original Contribution

c-Src deactivation by the polyphenol 3-*O*-caffeoylquinic acid abrogates reactive oxygen species-mediated glutamate release from microglia and neuronal excitotoxicity

Renato Socodato [a,*], Camila C. Portugal [a], Teresa Canedo [b], Ivan Domith [c], Nadia A. Oliveira [c], Roberto Paes-de-Carvalho [c,d], João B. Relvas [a], Marcelo Cossenza [c,e,**]

[a] Glial Cell Biology Laboratory Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal
[b] Addiction Biology Laboratory, Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150-180 Porto, Portugal
[c] Neurosciences Program, Fluminense Federal University, Niterói 24210-130, Brazil
[d] Department of Neurobiology, Institute of Biology, Fluminense Federal University, Niterói 24210-130, Brazil
[e] Department of Physiology and Pharmacology, Biomedical Institute, Fluminense Federal University, Niterói 24210-130, Brazil

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ABSTRACT

3-*O*-caffeoylquinic acid (3-CQA) is an isomer of chlorogenic acid, which has been shown to regulate lipopolysaccharide-induced tumor necrosis factor production in microglia. Whereas overactivation of microglia is associated with neuronal loss in brain diseases via reactive oxygen species (ROS) production and glutamate excitotoxicity, naïve (nonactivated) microglia are believed to generate little ROS under basal conditions, contributing to the modulation of synaptic activity and nerve tissue repair. However, the signaling pathways controlling basal ROS homeostasis in microglial cells are still poorly understood. Here we used time-lapse microscopy coupled with highly sensitive FRET biosensors (for detecting c-Src activation, ROS generation, and glutamate release) and lentivirus-mediated shRNA delivery to study the pathways involved in antioxidant-regulated ROS generation and how this associates with microglia-induced neuronal cell death. We report that 3-CQA abrogates the acquisition of an amoeboid morphology in microglia triggered by Aβ oligomers or the HIV Tat peptide. Moreover, 3-CQA deactivates c-Src tyrosine kinase and abrogates c-Src activation during proinflammatory microglia stimulation, which shuts off ROS production in these cells. Moreover, forced increment of c-Src catalytic activity by overexpressing an inducible c-Src heteromerization construct in microglia increases ROS production, abrogating the 3-CQA effects. Whereas oxidant (hydrogen peroxide) stimulation dramatically enhances glutamate release from microglia, such release is diminished by the 3-CQA inhibition of c-Src/ROS generation, significantly alleviating cell death in cultures from embryonic neurons. Overall, we provide further mechanistic insight into the modulation of ROS production in cortical microglia, indicating antioxidant-regulated c-Src function as a pathway for controlling microglia-triggered oxidative damage.

Introduction

Microglia are resident immune cells of the central nervous system (CNS) that are associated with neuroinflammation. Whereas resting microglial cells actively patrol the CNS parenchyma in vivo, modulating neuronal activity and synaptic remodeling [1], overactivation of microglia has been associated with triggering neuronal cell damage [2]. Among the bioactive substances released from overactivated microglia, reactive oxygen species (ROS) and glutamate have been anticipated to play prominent roles both in microglia-dependent neuronal loss and in the pathogenesis of neurodegenerative disorders such as Parkinson and Alzheimer diseases [3]. Although compelling evidence shows that several kinase pathways control the activation of microglial cells by divergent extracellular stimuli, the role played by protein tyrosine kinases in regulating ROS production and glutamate release in nonstimulated (naïve) microglia has not been fully appreciated. Moreover, interfering with ROS generation and/or glutamate release in naïve microglia (using antioxidants, for instance) might alleviate neuronal damage by preventing microglia overactivation.

Polyphenols are classic anti-inflammatory and antioxidant molecules. Chlorogenic acid (CGA) is a polyphenol compound naturally formed by the esterification of cinnamic acids with (−)-quinic acid, comprising a group of isomers (mainly 3-*O*-caffeoylquinic acid

* Corresponding author.
** Corresponding author at: Department of Physiology and Pharmacology, Biomedical Institute and Neurosciences Program, Fluminense Federal University, Niterói 24210-130, Brazil.
E-mail addresses: renato.socodato@gmail.com (R. Socodato), mcossenza@gmail.com (M. Cossenza).

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