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Resveratrol inhibits nitric oxide and TNF-α production by lipopolysaccharide-activated microglia

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Abstract

Upon activation, brain macrophages, the microglia, release proinflammatory mediators that play important roles in eliciting neuroinflammatory responses associated with neurodegenerative diseases. As resveratrol, an antioxidant component of grape, has been reported to exert anti-inflammatory activities on macrophages, we investigated its effects on the production of TNF-alpha (TNF-α) and nitric oxide (NO) by lipopolysaccharide (LPS)-activated microglia. Exposure of cultured rat cortical microglia and a mouse microglial cell line N9 to LPS increased their release of TNF-α and NO, which was significantly inhibited by resveratrol. Further studies revealed that resveratrol suppressed LPS-induced degradation of IκBα, expression of iNOS and phosphorylation of p38 mitogen-activated protein kinases (MAPKs) in N9 microglial cells. These results demonstrate a potent suppressive effect of resveratrol on proinflammatory responses of microglia, suggesting a therapeutic potential for this compound in neurodegenerative diseases accompanied by microglial activation.

Keywords: Resveratrol; Microglia; NO; TNF-α; IκBα; p38

1. Introduction

Resveratrol (*trans*-3, 4′ , 5-tri-hydroxystilbene), a polyphenol present in red wines and contained in various food components, possesses a variety of biological activities on macrophages. These include the inhibition of cyclooxygenase activity [1,2], the

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expression of integrins, the attachment to endothelial cells, and the generation of nitric oxide (NO) [3]. Resveratrol has also been shown to inhibit the release of TNF-alpha (TNF-α), IL-1β and IL-6 by lipopolysaccharide (LPS)-activated mice monocytes [4].

Microglia, a kind of cells of the macrophage lineage in the central nervous system (CNS), is quiescent in the normal brain. However, these cells can be activated by cytokines produced by infiltrating immune effector cells after CNS injury or by LPS during bacterial infection [5,6]. Activation of microglia is associated with increased phagocytosis and release of oxygen radicals, NO, proteases as well as pro-inflammatory cytokines [6,7]. Prolonged and excessive stimulation of microglia initiates an inflammatory cascade in the CNS that contributes to the pathogenesis of several neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) [6,8], multiple sclerosis [9] and HIV-associated dementia [10]. Thus, it is believed that agents that suppressing micrgolial cell activation are beneficial for the treatment of neurodegenerative diseases.

Based on the reported anti-inflammatory activity of resveratrol, the present study investigated the capacity and possible mechanism of this food component to inhibit microglia activation by proinflammatory stimulants.

2. Materials and methods

2.1. Materials

Resveratrol (purity 99.8%) was isolated from grape seeds and provided by Estacao Vitinicola Nacional, Instituto National de Investigacao Agraria, Portugal. The method for isolation of resveratrol was the same as that described previously [11–13]. Fetal bovine serum (FBS) was purchased from TBD Biotechnology Development (Tianjin, China); LPS (E5:055) and L-NAME (N_{ω}-nitrite-L-arginine-methyl-ester) were purchased from Sigma (St. Louis, MO, USA); Thiazolyl blue (MTT) was from Sino-American Biotechnology (Beijing, China); IκBα antibodies, p38 and phosphorylated p38 antibodies were from Santa Cruz Biotechnology (Santa Cruz, USA); iNOS antibody was purchased from Cayman Chemical (Ann Arbor, MI, USA); mTNF-α was from Diaclone (Besancon Cedex, France); Dulbecco's modified Eagle's medium (DMEM) and Iscove's modified dulbecco's medium (IMDM) were from Gibco (Grand Island, USA). Resveratrol was dissolved initially in dimethyl sulfoxide (DMSO) and was diluted with PBS for experiments. DMSO at the highest concentration possibly present in experimental conditions (0.1%) was not toxic to cells.

2.2. Cell culture

The murine microglial cell line N9 was a kind gift from Dr. P. Ricciardi-Castagnoli (Universita Degli Studi di Milano-Bicocca, Milan, Italy). The cells were grown in IMDM supplemented with 5% heat-inactivated FBS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5×10^{-5} M 2-mercaptoethanol.

Primary rat microglia were prepared from the cortical of newborn Wistar rats (1 day) [14]. Briefly, meninges and blood vessels of the rat cortex were removed. Tissues were dissociated with 0.25% trypsin at 37 °C for 15 min, and then the cell suspension was filtered through 50-μm diameter nylon meshes. The cells were collected by centrifugation at 1200 rpm/min for 10 min, re-suspended in DMEM supplemented with 10% FBS, then were plated in culture flasks. After 9–11 days, the flasks were shaken on a rotary shaker at 240 rev/min for 1 h. The resulting cell suspension rich in microglia was placed on culture dishes in which the cells adhered after 30 min at 37 °C. The purity of cells obtained was >95%.

Cells at density of 3×10^4 cells/well or 5×10^5 cells/cm^2 were plated onto 96-well microtiter plates or flasks for MTT, nitrite, TNF-α assay and Western blot analysis, respectively. Resveratrol (0.01–10 μg/ml) with or without LPS (1 μg/ml) were added to the culture medium of microglial cells for 48 h for the experiments.

2.3. Cell viability

Cell viability was evaluated by the MTT reduction assay [15]. In brief, cells were seeded in 96-well microtiter plates and treated with various