Supporting Information to:

Hydrophilic Ester-Bearing Chlorogenic Acid Binds to a Novel Domain to Inhibit Xanthine Oxidase

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Caffeic acid analogues inhibit nitric oxide production in LPS-activated cells

ROS have been viewed as general messengers for signaling pathways that are associated with key biochemical events during inflammation. It was reported that suppression of ROS-mediated nitric oxide (NO) elevation is beneficial for reducing the development of inflammation. Because blocking XO activity was sufficient to prevent NO production in our previous reports, nitrite production was used as an indicator of NO release in LPS-activated macrophages. Nitrite concentrations in culture media were measured with and without caffeic acid derivatives of co-incubated macrophages activated by lipopolysaccharide (LPS; 200 ng/mL). When LPS was administered to RAW 264.7 macrophages, NO production dramatically increased. The inhibition of NO release with respect to control LPS-activated macrophages co-incubated with caffeic acid, CAPE, and chlorogenic acid was determined. The results show that the agents reduced LPS-induced NO production in the following order: CAPE > caffeic acid ≥ chlorogenic acid (**Fig. 1S**). CAPE was the most potent NO-suppressive agent in LPS-treated macrophages, with 36.5% inhibition at a concentration of 0.5 μM. The cell viability assay verified that the inhibition was not due to general cellular toxicity. Chlorogenic acid displayed the weakest inhibitory effects in LPS-activated NO production, and this was speculated to be due to its hydrophilic ester, which contributes to lower cell membrane permeability.

Experimental method

NO_2^- accumulation was used as an indicator of NO production in the medium as described previously [16]. After different treatments, the culture supernatant of RAW 264.7 cells was mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% naphthylenediamine dihydrochloride, and 2% phosphoric acid) and incubated at room temperature for 15 min. Nitrite production was then determined by measuring the optical density at 540 nm.

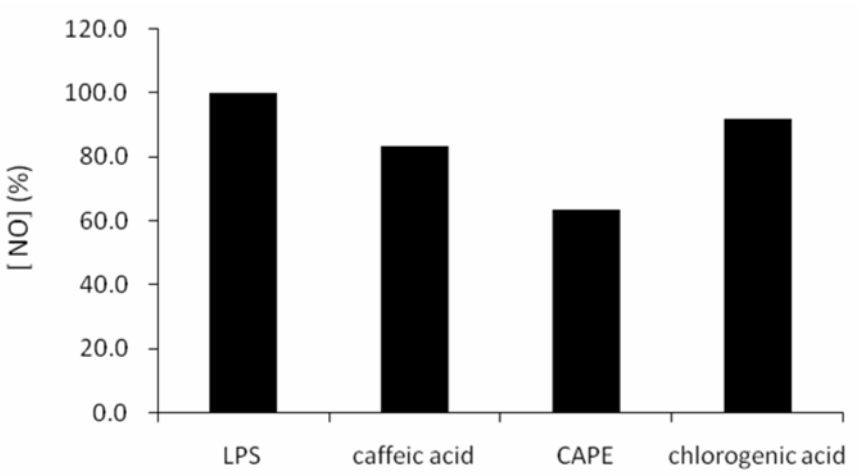

Fig. 1S Inhibition of NO production in LPS-activated macrophage cells by caffeic acid (10 μM), CAPE (0.5 μM), and chlorogenic acid (10 μM).