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Anti-neuroinflammatory and Anti-amyloidogenic Properties of Punicalagin in LPS-Activated Rat Primary Microglia

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Neuroinflammatory processes have been shown to have a fundamental role in the pathogenesis of neurodegenerative disorders like Alzheimer's disease (AD). Based on the evidence that inflammatory processes are involved in the pathogenesis of AD, research has been focusing on the potential therapeutic application of anti-inflammatory compounds in AD. Punicalagin is a polyphenolic active constituent of the pomegranate fruit, *Punica granatum*, which has shown promising anti-inflammatory activity in cancer and skin cells. However, nothing is known about the potential effects of this compound in neuroinflammation, and ultimately amyloid formation in the brain. We therefore evaluated the effects of different concentrations of punicalagin on LPS-induced neuroinflammation and amyloidogenesis in rat primary microglia. .Levels of pro-inflammatory cytokines in supernatants from LPS-activated primary microglia were measured using ELISA while PGE_2 released was measured using enzyme immunoassay (EIA), while nitrite production was measured with the Griess assay. Western blot analysis was used to evaluate the effects of punicalagin on protein expressions of COX-2, iNOS, mPGES-1, p38 MAPK and the β-site APP Cleaving Enzyme 1 (BACE-1) in LPS-activated microglia. Punicalagin (5-40 μM) produced significant ($p<0.05$) and dose-dependent reduction of PGE_2, nitrite, TNFα and IL-6 in microglia incubated with LPS for 24 hours. At the highest concentration tested (40 μM), punicalagin-treated cells produced 39.4±2.6% PGE_2, 35.7±3.9% nitrite, 32.0±3.0% TNFα and 43.3±2.1% IL-6, compared with control (LPS only) cells. Further investigations using immunoblotting revealed inhibition of COX-2, iNOS, mPGES-1, phospho-p38 and BACE-1 protein expressions in LPS-stimulated microglia. At a concentration of 40 μM, punicalagin pre-treatment resulted in 31.7±17.9% COX-2, 18.0±4.2% iNOS, 35.2±6.5% mPGES-1, and 26.0±5.1% BACE-1 protein expressions, compared with LPS control. Cell viability experiments using the MTT assay showed that punicalagin was not toxic to rat primary microglia at concentrations up to 40 μM. These observations indicate that punicalagin possesses both anti-neuroinflammatory and anti-amyloidogenic effects in LPS-activated rat primary microglia. It is suggested that punicalagin might be a potential therapeutic source for delaying the progression of AD.