Inhibition of NOS inhibitors and lipopolysaccharide induced inducible nitric oxide synthase and cyclooxygenase 2 gene expressions by rutin, quercetin, and quercetin pentaacetate in RAW 264.7 macrophages

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Abstract

Several natural flavonoids have been demonstrated to perform some beneficial biological activities, however, higher-effective concentrations and poor-absorptive efficacy in body of flavonoids blocked their practical applications. In the present study, we provided evidences to demonstrate that flavonoids rutin, quercetin, and its acetylated product quercetin pentaacetate were able to be used with nitric oxide synthase (NOS) inhibitors (N-nitro-L-arginine (NLA) or N-nitro-L-arginine methyl ester (L-NAME)) in treatment of lipopolysaccharide (LPS) induced nitric oxide (NO) and prostaglandin E2 (PGE2) productions, inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) gene expressions in a mouse macrophage cell line (RAW 264.7). Theresults showed that rutin, quercetin, and quercetin pentaacetate-inhibited LPS-induced NO production in a concentration-dependent manner without obvious cytotoxic effect on cells by MTT assay using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide as an indicator. Decrease of NO production by flavonoids was consistent with the inhibition on LPS-induced iNOS gene expression by western blotting. However, these compounds were unable to block iNOS enzyme activity by direct and indirect measurement on iNOS enzyme activity. Quercetin pentaacetate showed the obvious inhibition on LPS-induced PGE2 production and COX-2 gene expression and the inhibition was not result of suppression on COX-2 enzyme activity. Previous study demonstrated that decrease of NO production by L-arginine analogs effectively stimulated LPS-induced iNOS gene expression, and proposed that stimulatory effects on iNOS protein by NOS inhibitors might be harmful in treating sepsis. In this study, NLA or L-NAME treatment stimulated significantly on LPS-induced iNOS (but not COX-2) protein in RAW 264.7 cells which was inhibited by these three compounds. Quercetin

pentaacetate, but not quercetin and rutin, showed the strong inhibitory activity on PGE2 productionand COX-2 protein expression in NLA/LPS or L-NAME/LPS co-treated RAW 264.7 cells. These results indicated that combinatorial treatment of L-arginine analogs and flavonoid derivates, such as quercetin pentaacetate, effectively inhibited LPS-induced NO and PGE2 productions, at the same time, inhibited enhanced expressions of iNOS and COX-2 genes.