Piperine, a Primary Component of Black Pepper, inhibits Prostagladins Generation by Suppression of COX Activity on Arachidonic Acid Metabolism in LPS-Stimulated RAW264.7 Cells

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Piperine (piperinoyl-piperidine) is a nitrogenous pungent substance contained in black pepper, the well know spice obtained from *Piper nigrum* L. (*Piperaceae*). Pharmacological studies have shown that piperine reduces inflammation and pain, possesses anticonvulsant and antiulcer activity, protects the liver and has deleterious effects on testis function. Prostaglandins(PGs) are a family of intercellular and intracellular messengers derived from arachidonic acid(AA) by phospholipase(PL) and cyclooxygenase(COX). These mediators exert a wide range of effects on processes such as smooth muscle tone, vascular permeability, cellular proliferation, and inflammatory/immune function. In this study, Piperine, a primary component of black pepper, potently reduced the generations of PGE_2 and PGD_2 in RAW264.7 cells stimulated by lipopolysaccharide(LPS) in a dose-dependent manner when added to the culture media at the time of stimulation. In order to elucidate the mechanism involved in the anti-inflammatory activity of Piperine, we investigated its effects on the AA metabolism and enzyme activity such as $cPLA_2$-, $sPLA_2$- and COX-activity, and protein expression such as $cPLA_2$- and COX_2-expression. In the results, LPS stimulated the generations of PGE_2 and PGD_2 in RAW264.7 cells in a dose- and time-dependent manner. Piperine inhibited the COX activity, but did not suppress the $cPLA_2$-, or $sPLA_2$-activity in LPS-stimulated RAW264.7 cells. Furthermore, Piperine did not affect the $cPLA_2$-, or COX_2 expression. These results suggest that Piperine may inhibit the generations of PGE_2 and PGD_2 in LPS-stimulated RAW264.7 cells probably through the suppression of the COX activity in LPS-stimulated RAW264.7 cells.

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Mechanism of immunostimulating action of polysaccharide isolated from Platycodon grandiflorum in RAW 264.7 macrophages

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In our previous study, we reported that PG, a polysaccharide isolated from Plyatycodon grandiflorum, activated macrophages and B cells, but not T cells. Here, we investigated in more detail the mechanism of action of PG in macrophage activation. Since PG cannot penetrate cells due to the large molecular mass, it should bind to membrane receptors of macrophages. We showed that some antibodies to cell surface molecules (CD14, CD11b, TLR2, and TLR4) inhibited RAW264.7 macrophage activation, suggesting the possible binding sites of PG. The role of TLR4 as the PG receptor was also confirmed by the results that PG activity in macrophages from C3H/HeJ, known to have a defective TLR4, was completely inhibited. Ligation of TLR2/4 by PG also resulted in the activation of JNK, p38 and ERK1/2 MAPKs, which was examined by immunoblotting and kinase assays. It also resulted in the phosphorylation of IkBs, the translocation NF-kB into the nucleus and the initiation of gene transcriptions of IL-1b, IL-6, TNF-a and iNOS. In spite of the similarity in their mode of action, PG and LPS were differentiated by using polymyxin B, which only inhibited macrophage activation by LPS, but not PG. Taken together, our results indicated that PG, as a plant-derived polysaccharide, activated macrophages by mediating TLR signaling cascades and might accelerate the innate immunity against to infectious pathogens and cancers.