2', 3-dihydroxy-5-methoxybiphenyl suppresses fMLP-induced superoxide anion production and cathepsin G release by targeting the beta-subunit of G-protein in human neutrophils

Hsiang Ruei Liao^{1,2,4}, Ih-Sheng Chen³, Fu-Chao Liu³, Shinn-Zhi Lin¹, Ching-Ping Tseng^{4,5,6}

¹Graduate Institute of Natural Products, Chang-Gung University, Taiwan, ²Department of Anesthesiology, Chang Gung Memorial Hospital, Taiwan, ³Graduate Institute of Natural Products, College of Pharmacy, Kaohsiung Medical University, Taiwan, ⁴Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taiwan, ⁵Department of Medical Biotechnology and Laboratory Science, College of Medicine, Chang Gung University, Taiwan, ⁶Department of Laboratory Medicine, Chang Gung Memorial Hospital, Taiwan

This study investigates the effect and the underlying mechanism of 2',3-dihydroxy-5-methoxybiphenyl (RIR-2), a lignan extracted from the roots of *Rhaphiolepis indica* (L.) Lindl. ex Ker var. *tashiroi* Hayata ex Matsum. & Hayata (Rosaceae), on N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP)-induced respiratory burst and cathepsin G in human neutrophils. However, 2',3-dihydroxy-5-methoxybiphenyl (RIR-2) was not found to inhibit PMA-induced respiratory burst and cathepsin G release. Signaling pathways regulated by RIR2 which modulated fMLP-induced respiratory burst and cathepsin G release were evaluated by an interaction between G β -protein with downstream signaling induced by fMLP and by immunoblotting analysis of the downstream targets of G β -protein. Briefly, 2',3-dihydroxy-5-methoxybiphenyl (RIR-2) inhibited fMLP-induced superoxide anion production ($IC_{50}=2.57\pm0.22\ \mu M$, $n=4$), cathepsin G release ($IC_{50}=18.72\pm3.76\ \mu M$, $n=4$) and migration in a concentration dependent manner. Further, 2',3-dihydroxy-5-methoxybiphenyl (RIR-2) specifically suppresses fMLP-induced Src family kinases phosphorylation, -Raf/ERK phosphorylation, by inhibiting an interaction between G β -protein with Src kinases without inhibiting Src kinases activities. Consequently, 2',3-dihydroxy-5-methoxybiphenyl (RIR2) attenuated the downstream targets of Src kinase, such as Tec translocation from the cytosol to the inner leaflet of the plasma membrane, phosphorylation of AKT, P38, PLC γ 2, PKC and membrane localization of p47phox and P40phox, furthermore, 2',3-dihydroxy-5-methoxybiphenyl (RIR-2) attenuated fMLP-induced intracellular calcium mobilization, moreover, 2',3-dihydroxy-5-methoxybiphenyl (RIR-2) was not found to increase cyclicAMP. 2',3-dihydroxy-5-methoxybiphenyl (RIR2) was not a competitive or allosteric antagonist of fMLP. On the other hand, PMA-induced phosphorylation of Src, AKT, P38, PKC and membrane localization of p47phox and P40phox remained unaffected. In conclusion, 2',3-dihydroxy-5-methoxybiphenyl (RIR2) specifically modulates fMLP-mediated neutrophil superoxide anion production and cathepsin G release by inhibiting an interaction between G β -protein with downstream signaling which subsequently interferes with the activation of intracellular calcium, PLC γ 2, AKT, p38, PKC, ERK, p47phox and p40phox.