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Original article

Dipeptide HCH6-1 inhibits neutrophil activation and protects against acute lung injury by blocking FPR1

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ABSTRACT

Formyl peptide receptor 1 (FPR1) is an emerging therapeutic target for the discovery of drugs to treat neutrophilic inflammatory diseases. However, development of FPR1 antagonists for clinical use is still inadequate. The purpose of this study was to identify a synthetic dipeptide *N*-(*N*-benzoyl-L-tryptophanyl)-D-phenylanlanine methyl ester (HCH6-1) as a FPR1 inhibitor and to investigate its protective effects against acute lung injury (ALI). HCH6-1 inhibited superoxide anion generation, elastase release, and chemotaxis in human neutrophils specifically activated by formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLF), an FPR1 agonist. HCH6-1 produced right shifts in the concentration-response curves of fMLF, suggesting that HCH6-1 was a competitive antagonist of FPR1. Indeed, HCH6-1 bound to FPR1 in human neutrophils and neutrophil-like THP-1 as well as hFPR1-transfected HEK293 cells. Also, the FPR1 downstream signaling pathways were competitively inhibited by HCH6-1. Furthermore, HCH6-1 prevented pulmonary neutrophil infiltration and edema along with alveolar damage in LPS-induced ALI in mice. Our findings suggest that HCH6-1, a FPR1 antagonist, may have potential as a new therapeutic agent for treating FPR1-involved inflammatory lung diseases.

1. Introduction

During the inflammatory process, neutrophils are recruited into inflammatory areas to eliminate invasive pathogens [1]. However, excessive recruitment and activation of neutrophils are harmful to human health and are involved in the progression of acute and chronic inflammatory diseases, including acute lung injury (ALI), rheumatoid arthritis, and sepsis [2–4]. Superoxide anion and proteolytic enzymes released from activated neutrophils have been proposed to facilitate tissue injury [5,6]. The recruitment of activated neutrophils into the lung can cause endothelial damage and further enhance permeability of the alveolar-capillary barrier in ALI or acute respiratory distress syndrome [7,8]. Mortality in ALI patients remains high despite several treatment strategies [9,10].

Formyl peptide receptor 1 (FPR1) mediates neutrophil activation in response to bacterial or mitochondrial formyl peptides stimulation [11–14]. FPR1 typically facilitates neutrophil immune responses in the presence of *N*-formyl peptides derived from bacteria in vitro [12,15,16]. Recent data suggested that *N*-formyl peptides are also considered damage-associated molecular patterns that trigger sterile inflammatory responses through FPR1-mediated responses [2,17–19]. Mitochondrial lysates from trauma-damaged tissue recruit neutrophils to induce severe inflammatory response syndrome via activation of FPR1 [20]. In addition, hepatocyte death is amplified by liver neu-

Abbreviations: $[Ca^{2+}]_i$, intracellular calcium concentration; ALI, acute lung injury; CB, cytochalasin B; CsH, cyclosporine H; FBS, fetal bovine serum; FNLFNYK, *N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys-fluorescein; FPR1, formyl peptide receptor 1; HE, Hydroethidine; H89, N-[2-(*p*-bromocinnamylamino)ethyl] − 5-isoquinolinesulfonamide; HCH6-1, *N*-(*N*-benzoyl-L-tryptophanyl)-D-phenylanlanine methyl ester; LDH, lactate dehydrogenase; LTB4, leukotriene B4; MMK1, Leu-Glu-Ser-Ile-Phe-Arg-Ser-Leu-Leu-Phe-Arg-Val-Met; NMR, nuclear magnetic resonance; PKA, protein kinase A; THP-1 cells, human monocytic leukemia cells; WKYMVm, Trp-Lys-Tyr-Met-Val-D-Met; WST-1, 2-(4-Iodophenyl) − 3-(4-nitrophenyl) − 5-(2,4-disulfophenyl) − 2H-tetrazolium monosodium salt; WRW4, Trp-Arg-Trp-Trp-Trp-Trp-CONH2

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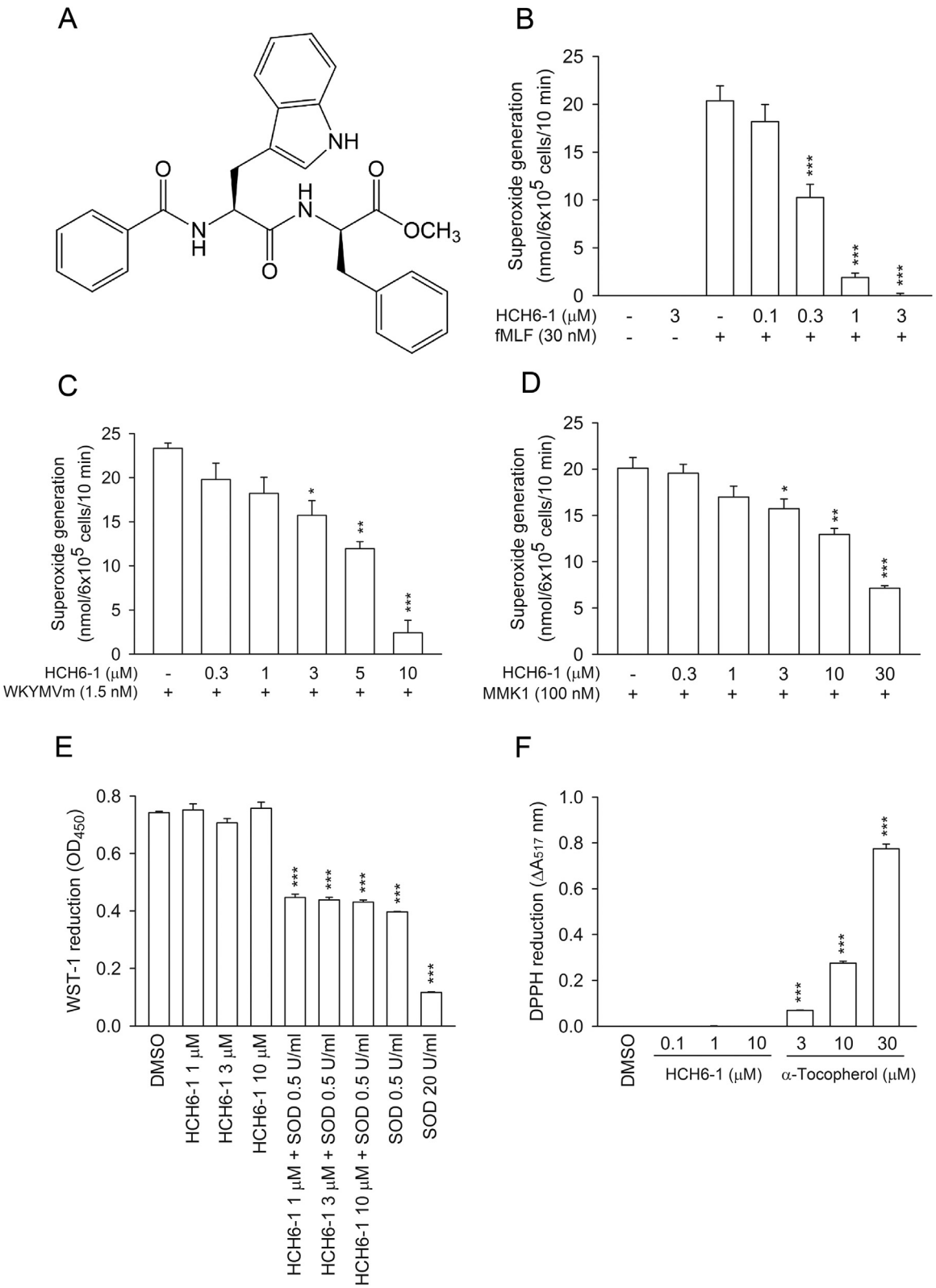

Fig. 1. HCH6-1 selectively inhibits extracellular superoxide anion generation in FPR1-agonist stimulated human neutrophils. (A) The chemical structure of HCH6-1. Human neutrophils were incubated with DMSO (as the control) or HCH6-1 for 5 min, and then activated by (B) fMLF, (C) WKYMVm or (D) MMK1 in the presence of CB for another 10 min. Superoxide anion generation was measured by cytochrome *c* reduction by spectrophotography. Reduction of (E) WST-1 and (F) DPPH level was measured at 450 nm and 517 nm, respectively. Data are mean ± SEM. (n = 4 for B and C, n = 8 for D, n = 3 for E and F). $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ compared with the control.

trophil infiltration, and the release of mitochondrial products into the circulation may induce a systemic inflammatory response and further trigger lung injury [21]. Therefore, determining how to modulate FPR1 functions on immune responses is crucial.

Aurantiamide acetate and its analogs from *Polygonum chinensis Linn* have various biological activities. A series of dipeptide analogs of aurantiamide acetate exhibited inhibitory effects on superoxide anion generation and elastase release in formyl-L-methionyl-L-leucyl-L-pheny-