STUDY OF SYNTHETIC PEPTIDES DERIVED FROM PKI55, A PKC MODULATOR, IN STIMULATED HUMAN NEUTROPHILS

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For-Met-Leu-Phe-OH (fMLP) and its derivative methyl ester, fMLP-OMe, represent highly potent chemoattractants for neutrophils. The interaction of fMLP with its receptor (FPR) activates multiple second messengers and involves specific kinases such as protein kinase C (PKC) and mitogen activated protein kinases (MAPKs). We reported a strong relationship between specific PKC isoforms and human neutrophils function activated by formylpeptides [1]. Recently, we have identified the PKI55 protein that acts as a specific modulator of PKC [2]. We tested peptides derived from both C-terminal and N-terminal sequence of PKI55, with the objective to identify the portion of the protein maintaining the biochemical effect to inhibit PKC. Enzyme activity *in vitro* assay, using recombinant PKC isoforms, showed that the peptides G16, G8 and G5 (Tab.1), inhibited the specific PKC isozymes. These same peptides were used to evaluate in human neutrophils their ability to affect chemotaxis, superoxide and lysozyme release. Neutrophils were purified from peripheral blood of health donors, preincubated with peptides at concentration from 0,1μM, to 25μM and then activated with fMLP-OMe. Our data demonstrate that the peptides reduced chemotaxis, while superoxide generation and lysozyme release were never modified. Previously we demonstrated that chemotactic movement of neutrophils was mediated by activation of PKC β_1 [3]. Since we suggest that the selected peptides act as PKC inhibitors, to confirm our hypothesis western blotting analysis were performed on neutrophils stimulated with fMLP-OMe in presence or absence of G16, G8 and G5 and PKC β_1 levels were studied. The results confirmed the significant reduction of PKC β_1 levels in presence of G16, G8 and G5 peptides in comparison with control samples. The peculiar inhibiting properties of the peptides render them a promising pharmacological tool to control the over-expression of PKC isoforms.

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[2] Selvatici R., Melloni E., Ferrati m., Piubello C., Marincola F.C. And Gandini (2003) E. J. Mol Evol, 57:131-139. [3] Spisani S., Falzarano S., Traniello S., Nalli M. and Selvatici R. (2005) FEBS J. 272:883-91.

Table 1.

Peptide	***Aminoacid sequences***
PKI55	MLYKLHDVCRQLWFSCPACHHRAMRICCPAQHHRTISVCKTILSSPPPLDSLPCM
G16	MLYKLHDVCRQLWFSC
G8	CRQLWFSC
G5	CRQLW