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Influence of polymer polypeptides and IL-6R peptides on chemotaxis of the macrophage-like cell line J774

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Introduction

Polylysine based branched chain polymeric polypeptides are often used as macromolecular carriers of drugs and epitopes. Charge and hidrophobicity of the amino acid components of their side-chains influence the biological properties like cytotoxicity, biodistribution or immunogenicity [1,2].
Chemotaxis is an essential biological function in the living organism. For example the migration of monocytes is an important step in the activation of the innate immunity. Chemotactic activity of different cell types is often induced by oligopeptides [3]. Our previous investigations showed that some peptides corresponding to the SXWS sequence are chemoattractant to the unicellular *Tetrahymena pyriformis* GL [4]. The WSXWS sequence is present in the family of the hematopoetic cytokin receptors including interleukin-6 receptor. In the position X, various amino acids (in the case of IL-6R, Glu or Asp) can be found.
The chemotactic properties of the branched chain polymeric polypeptides with the general formula poly[Lys(X_i)], (X_iK), where X=His, Pro and Glu and poly[Lys(X_i-DL-Ala_m)], (XAK) where X=Thr and Ac-Glu have also been examined on *Tetrahymena pyriformis*. We found that polymer H_iK, (poly[Lys($His_{0.56}$)] elicited pronounced positive chemotaxis [5].

Results and Discussion

The aim of this study was to establish structure-chemotactic properties correlation in a group of branched chain polypeptides and also in a group of IL-6R peptides (SXWS, WSXWS) in the murine monocyte-macrophage cell-line J774.
First the cytotoxicity of ten polymeric polypeptides with different side-chain composition was analysed by MTT assay. We found that two polymers (poly[Lys] and H_iK) were toxic (88±0.14% and 82±1.8%, respectively) above 10 μg/ml concentration after 1 hour incubation. After 24 and 48 hour treatment P_iK, poly[Lys($Ser_{0.9}$-DL-$Ala_{4.0}$)] (SAK) and poly[Lys(Succ-$Glu_{1.0}$-DL-$Ala_{4.0}$)] (Succ-EAK) exhibited some toxicity (~ 20%).
In the second set of experiments the chemotactic activity of these polypeptides and IL-6R peptides was investigated in 96-well NeuroProbe® chamber. The polycationic poly[Lys] (0.02 and 0.2 μg/ml) as well as polyanionic Succ-EAK (0.02 μg/ml) elicited positive chemotaxis. Polypeptides SAK (0.02-20 μg/ml), TAK (0.02-0.2 μg/ml) and AK (poly[Lys($Thr_{1.0}$-DL-$Ala_{3.1}$)]) (0.02-20 μg/ml) containing long, positively charged side chains were also chemoattractant. Polypeptide P_iK was repellent at the highest concentration studied (20 μg/ml).
Six SXWS/WSXWS peptide pairs including amino acid X with different character were selected (X=E, K, F, T, V and L). Peptide SXWS containing Phe in position X proved to be chemoattractant in a wide concentration range (Figure 1). Peptides SKWS ($c=10^{-10}$M), STWS ($c=10^{-6}$-10^{-8}M) and SLWS ($c=10^{-12}$M) were slightly repellent. No chemotactic activity was observed with peptides SEWS and SVWS. Taken together in the case of the

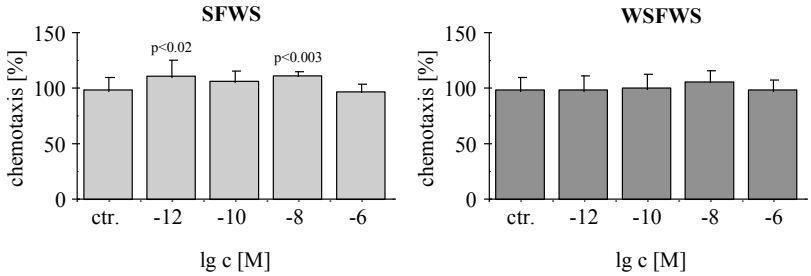

Fig. 1. The effect of peptides SFWS and WSFWS on the chemotaxis of J774 cells

SXWS peptides we found that the character of the amino acid X influences the chemotaxis of J774 cells. Peptide WSEWS comprising the native IL-6 receptor sequence elicited a weak positive chemotaxis at 10^{-8}M concentration. Other WSXWS pentapeptides, in contrast to their tetrapeptide counterparts, did not induce chemotaxis of the J774 cells, except for WSTWS at 10^{-6}M concentration.

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