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IN VITRO EVALUATION OF NOVEL IN-111 LABELED CXCR4 ANTAGONISTS

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BACKGROUND-AIM

The CXCR4/CXCL12 axis plays an important role in cancer metastases. CXCR4 inhibitors are being evaluated for anticancer therapy as well as for the development of imaging agents. Recent work from our institution has identified small peptide molecules containing a three amino acid motif (Ar-Ar-X or X-Ar-Ar) that inhibit CXCR4 dependent cell migration and the formation of lung metastases in animal models. Peptide R (Arg-Ala-[Cys-Arg-Phe-Phe-Cys]) and peptide S (Arg-Ala-[Cys-Arg-His-Trp-Cys]) have shown the best CXCR4 inhibition properties.We are evaluating binding properties of DTPA coupled derivatives of peptides R and S for use as radiopharmaceuticals for targeting CXCR4 receptors in vivo.

METHODS

Peptides containing different spacers to distance the chelators from the active molecule were synthesized. Four derivatives of peptide R (DTPA-R, DTPA-Dioxa-R, DTPA-Dioxa3-R and DTPA-PEG-R) and one derivative of peptide S (DTPA-Ahoh-S) were coupled on the N-terminus. R-bAla-DTPA and S-bAla-DTPA were coupled on the C terminus. Binding inhibition by the new peptides of the PE-labeled anti-CXCR4 12G5 antibody was assessed by flow cytometry. Displacement experiments were performed with cold peptides against the high affinity CXCR4 ligands 125I SDF1alpha or 111In-DTPA-T140 on CEM lymphoblastic leukemia cells. All peptides were labeled with 111In in citrate buffer for saturation binding experiments.

RESULTS

All R and S peptide derivatives showed IC50 between in the 10-100 µM range in flow cytometry experiments, whereas the native peptides under the same conditions showed better inhibition (IC50 in the 1-10 µM range). Displacement experiments performed against SDF1alpha/CXCL12 or labeled T140 peptide showed no significant displacement of radioactivity of all new derivatives tested at concentrations up to 10 µM. Similarly, saturation binding experiments performed with 111In labeled peptides showed no saturable binding up to 100 µM with the exception of R-bAla-DTPA where saturable binding was obtained with a dissociation constant in the order of 10 µM.

CONCLUSION

All derivatives tested show affinities too low to be assessed with the described radio assays. The derivative of the R peptide containing the chelator at the C terminus did show saturable binding although with very low affinity. While the currently described derivatives may be of little value for imaging CXCR4 in vivo the R-bAla-DTPA derivative may be a starting point for development of higher affinity CXCR4 ligands based on these peptide sequences.