Effect of modification of the physicochemical properties of ICAM-1-derived peptides on internalization and intracellular distribution in the human leukemic cell line HL-60

ABSTRACT

The objective of this work is to test the hypothesis that increasing the hydrophilicity of DOX−peptide conjugates may modify their entry mechanisms into HL-60 cells from passive diffusion to receptor-mediated uptake. To test this hypothesis, the entry mechanisms and the intracellular disposition of DOX−cIBR7, DOX−PEGcIBR7, FITC−cIBR, and FITC−cIBR7 were evaluated in HL-60 cells. To increase the hydrophilicity of the peptide, the cIBR peptide (cyclo(1,12)PenPRGGSVLVTGC) was modified to cIBR7 peptide (cyclo(1,8)CPRGGSVC) by removing the hydrophobic residues at the C-terminus. DOX−cIBR7 conjugate, which has higher hydrophilicity than DOX−cIBR, was synthesized. Second, a hydrophilic linker (11-amino-3,6,9-trioxaundecanate linker) was incorporated between DOX and cIBR7 to generate DOX−PEGcIBR7 with higher hydrophilicity than DOX−cIBR7. As controls, FITC−cIBR and FITC−cIBR7 were used to check for any endocytic uptake process of the peptide. As previously found with DOX−cIBR, DOX−cIBR7, and DOX−PEGcIBR7, conjugates enter the cells via passive diffusion and not via the energy-dependent endocytic process. This result suggests that an increase in hydrophilicity does not influence the entry mechanism of the DOX−peptide conjugates. In contrast to the DOX−cIBR7 conjugate, the FITC−cIBR7 conjugate showed energy-dependent cellular entry into the cells and followed an endocytic pathway similar to that for dextran. Finally, the entry of DOX−cIBR7 and DOX−PEGcIBR into the cell cytosol was shown to be due to the properties of DOX and not to those of the peptide.

Keyword: ICAM-1 peptide; LFA-1; Endocytosis; Doxorubicin; Drug conjugate; HL-60 cells