

Performance of oligoarginine-linked polymers physically mixed with poorly membrane-permeable molecules on cell membranes

Shinji Sakuma

Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Setsunan University

We have been investigating cell-penetrating peptide-linked polymers as a new class of penetration enhancers. Cell-penetrating peptides are cationic oligopeptides such as oligoarginine and penetratin and they are internalized into cells via macropinocytosis. We designed a novel polymer: poly (N-vinylacetamide-co-acrylic acid) (PNVA-co-AA) modified with oligoarginine, with the expectation that the polymers would enable poorly membrane-permeable molecules physically mixed with them to effectively penetrate cell membranes without their concomitant cellular uptake. Here, we evaluated the performance of D-octaarginine-linked PNVA-co-AA on cell membranes. When cultured Caco-2 cells were incubated with 5(6)-carboxyfluorescein (CF), about 0.1% of applied CF was internalized into the cells during a 30-min experiment. This poor membrane permeability was dramatically enhanced by the D-octaarginine-linked polymers. A similar enhancement was observed when anionic CF was substituted with cationic atenolol and nonionic FITC-dextran. None of the individual components had any influence on CF uptake, demonstrating that only D-octaarginine anchored chemically to the polymeric platform enhanced the membrane permeation of CF. The polymer-enhanced CF uptake was consistently high even when the incubation time was extended to 120 min. Confocal laser scanning microphotographs of cells incubated with D-octaarginine-linked polymers bearing rhodamine red demonstrated that the cell outline was stained with red fluorescence. The polymer-enhanced CF uptake was significantly suppressed by 5-(N-ethyl-N-isopropyl) amiloride, which is an inhibitor of macropinocytosis. Results indicated that D-octaarginine-linked polymers remained on the cell membrane and poorly membrane-permeable molecules were continuously internalized into cells mainly via macropinocytosis repeated for the individual peptidyl branches in the polymer backbone without the concomitant cellular uptake of the polymers.