Direct Cytosolic Delivery of Molecular Imaging Agents Using Covalent Conjugates of Pyrenebutyrate

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Introduction:
The hydrophobic nature of the lipid bilayer makes it impossible for a vast variety of proteins and peptides to cross the plasma membrane. Exceptions to this rule are membrane transducing peptides commonly known as cell penetrating peptides (CPPs). These short cationic peptides have been extensively used in the last years to deliver various exogenous cargos into cells.
A better understanding of the mechanism of internalization for CPPs revealed primarily endocytotic uptake. Thus, the confinement of internalized biomolecules into endosomes becomes evident. In 2006, Takeuchi *et al*. came up with an interesting approach of co-incubating CPP with the counteranion pyrenebutyrate (PB) to obtain rapid cytosolic delivery (1). But this strategy, like most noncovalent co-incubation methods, not only works best with molar excess of PB but is also reported to be not applicable in the presence of medium or serum.
In this study, we attempt to improve over the above approach by covalently coupling PB with the CPP attached to (Gd)DOTA (a MRI agent) and fluorescein isothiocyanate (FITC) for MR and optical imaging as well.

Methods:
Cell experiments were performed with NIH-3T3 mouse fibroblasts cultured in 96 well microplates. Cells were incubated for different time points in the presence of chemically coupled dTat-FITC-PB, dTat-(Gd)DOTA-FITC-PB or a 1:1 mixture of free PB with free dTat-(Gd)DOTA-FITC.
After incubation, cell nuclei were counterstained with Hoechst 33342 and external fluorescence was quenched with trypan blue. Following repeated buffer washings, cell-related FITC fluorescence and cell number were evaluated in a multiplate reader. Subsequently, fluorescence microscopy was performed with the same cells to observe the cellular localization.

Results:
We observed a direct cytosolic uptake of covalent conjugates into cells within a few minutes. Only a low micromolar concentration of the applied compound was enough, which is a factor 4-5 less than what is needed for internalization with dTat alone. However, endocytotic mechanism takes over when the duration of incubation with compound is increased to 18 hrs. Our results indicate that PB only transiently delivers into cytosol while on longer incubations, endocytosis dominates. No cytotoxic effect was observed on cells at the used concentration even after long term incubation.

Conclusion:
The results show that covalently bound PB can transiently deliver cargoes with high efficacy into the cytosol and thus might help to overcome the complete endosomal trapping of CPP coupled probes for Molecular Imaging.

References
[1] T. Takeuchi *et al.*, *ACS chemical biology*, **1(5)** (2006) 299.