



ABSTRACT FORM

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Email : CDTM2008@cardiff.ac.uk

Abstract Details

Authors : Rajendra Joshi, Wu Su, Ritu Mishra, Joern Engelmann

Title of abstract: Evaluation of Cholesterol Conjugation to Antisense PNA for Cellular Delivery

Full Affiliation Address Max Planck Institute for Biological Cybernetics, Tuebingen, Germany

Telephone: +49 (0) 7071601705

Fax: +49 (0) 7071601702

Email: rajendra.joshi@tuebingen.mpg.de

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Introduction: Peptide nucleic acids (PNAs) are promising tools for antisense therapy in cells, as they present several advantages like high biological stability and binding of RNA and DNA targets in a sequence-specific manner. However, the main hindrance to the effective use of PNA has been their relatively poor uptake by cells. Many reports have already been published on the progress made in the development of cell penetrating peptide (CPP) based delivery systems. However, endosomal capture seems to be a major challenge in these approaches and the uptake mechanism might also be governed by the size of the molecule. Endosomal release or direct uptake of such agents into the cytosol is a prerequisite for efficient mRNA based targeting. Otherwise, it has recently been reported that covalent conjugates of cholesterol and siRNAs transported via lipoprotein particles were facilitating cellular import [1]. The aim of this study was to synthesize a cholesterol coupled antisense PNA and to compare its uptake characteristics with a CPP-PNA conjugate.

Methods: The synthesis of PNA (anti-dsRed PNA (agcgctgtacc), specifically targeted to mRNA of dsRed) conjugated to CPP (d-Tat) or cholesterol was performed in a fully automated synthesizer using continuous solid phase chemistry. Cellular uptake of compounds was confirmed by fluorescence microscopy and spectroscopy.

Results: PNA conjugated to CPP or cholesterol was synthesized and characterized by ESI-MS. Cell uptake studies showed that the CPP bound PNA was located predominantly in vesicles indicating an endosomal uptake mechanism and subsequent entrapment in vesicles. This might be favored by the relative large molecular size. The relatively small cholesterol bound PNA was also efficiently internalized. However, it was again located inside vesicles without detectable cytosolic distribution. This might be enhanced by saturating the conjugate with HDL or LDL particles. The solubility in water has also to be improved by increasing the availability of hydrophilic groups in the molecule.

Conclusion: PNA-Cholesterol has fewer coupling steps during synthesis and modest molecular size as compared to PNA-CPP. However, it was also taken up into cells by a predominantly endosomal mechanism restricting its applicability for mRNA targeting. The efficient uptake might make it a promising cellular delivery agent after further improvements.

[1] C. Wolfrum et al., Nature Biotechnology, 2007, 25, 1149