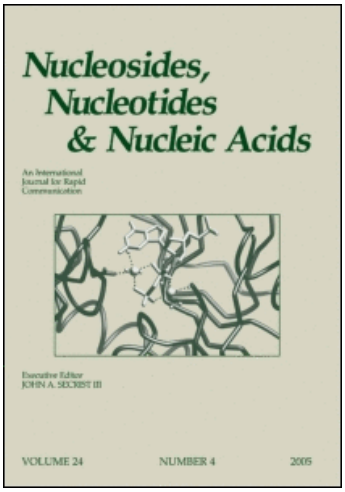

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Uptake and Intracellular Distribution of Oligonucleotides Vectorized by a PAMAM Dendrimer

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UPTAKE AND INTRACELLULAR DISTRIBUTION OF OLIGONUCLEOTIDES VECTORIZED BY A PAMAM DENDRIMER

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ABSTRACT : We studied the uptake and intracellular distribution of an FITC labelled phosphodiester oligodeoxynucleotide (ODN) vectorized by a dendrimeric structure in cell culture.

INTRODUCTION

Factors limiting the use of antisense ODNs are inefficient cellular uptake and intracellular transport to RNA targets[1]. A great number of chemical modifications have been proposed to increase ODN efficiency but they have raised problems related to ODN-sequence-specificity, stability of the ODN/RNA duplex and ODN abilility to activate RNase H[2]. Another means to enhance ODN activity is to vectorize them. We investigated, by flow cytometry, the ability of a polyamidoamine (PAMAM) dendrimer[3] to enhance cellular uptake of both 3'- and 5'-FITC labelled 18-mer phosphodiester ODN on different cell lines. We also studied, by confocal microscopy, the intracellular distribution of the vectorized FITC-ODN.

RESULTS AND DISCUSSION

Flow cytometry showed that complexation of the FITC-ODN to a PAMAM dendrimer (SuperFect™, Qiagen) strongly increased cellular fluorescence intensity which was greater on HeLa (human epitheloid carcinoma) and NIH 3T3 (murine fibroblast) cells compared

to CEM (nonadherent human lymphocyte) cells. Interestingly, a small population of cells (5 to 10 % of all the fluorescent cells) exhibited a strong intensity of fluorescence, at least 10 times higher than the intensity of the majority of fluorescent cells. After 30 minutes, fluorescent HeLa cells could already be detected and their percentage increased progressively during the first 4 h reaching a plateau of 80 to 90%. The cytotoxicity of the complex ODN-SuperFect™ was found to be insignificant on HeLa cells and approximately 30% on NIH 3T3. Confocal microscopy showed, with both adherent cell lines, an heterogeneity of the intracellular distribution of fluorescence whatever the time of incubation with the vectorized FITC-ODN (from 1 h to 24 h). Some cells exhibited perinuclear fluorescence and others, strong nuclear fluorescence. Controls carried out on cells with FITC alone and the analysis of FITC-ODN extracted from cells by PAGE showed that the fluorescence observed inside cells corresponded to the FITC-ODN intact.

In conclusion, we found that the dendrimeric structure permits the FITC-ODN to enter efficiently into cells. Moreover, in order to optimize ODN activity, it appears relevant to study in depth of the subpopulation of cells, observed by flow cytometry, presenting a high fluorescence intensity and the one, detected by confocal microscopy, showing a nuclear localization of FITC-ODN[4].

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