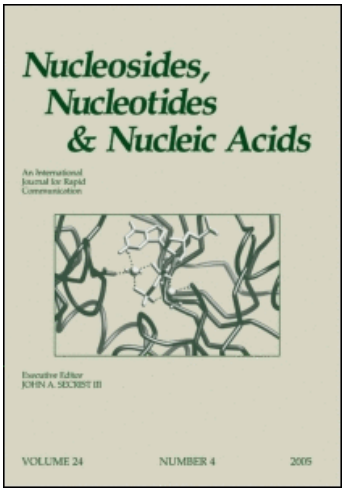

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CYTOTOXICITY AND IN-VIVO TOLERANCE OF FdUMP[10]: A NOVEL PRO-DRUG OF THE TS INHIBITORY NUCLEOTIDE FdUMP

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ABSTRACT: The cytotoxicity of the 10mer ODN FdUMP[10] towards human colorectal tumor cells was evaluated using a clonogenic assay. FdUMP[10] was more than 100-fold more active than 5-FU at inhibiting colony formation of H630 cells. FdUMP[10] was also evaluated for cytotoxicity in the NCI 60 cell line screen, and showed markedly improved activity relative to 5-FU against numerous tumor cell lines. The *in-vivo* tolerance of FdUMP[10] is more than three-fold greater per mole fluorinated pyrimidine, than 5-FU.

5-Fluorouracil (5-FU) is among the antimetabolite class of anticancer drugs and requires metabolic activation from the nucleobase to the 2'-deoxynucleoside-5'-O-monphosphate form (FdUMP) in order to inhibit thymidylate synthase (TS), the principal cellular target of 5-FU chemotherapy. Although chemotherapy with 5-FU remains a standard component in the treatment of various human malignancies, including breast and ovarian cancer, and especially colorectal cancer, 5-FU administration suffers from numerous drawbacks including dose-limiting toxicities and the frequent occurrence of cellular resistance. We have designed multimeric forms of FdUMP which are oligodeoxynucleotides in which 5-FU is the only nucleobase. The conceptualized mode of action of these compounds involves three steps: 1) Cellular uptake of FdUMP[N]; 2) 3' -> 5' exonucleolytic activity to release N FdUMP molecules from each molecule of FdUMP[N]; and 3) Inhibition of TS by FdUMP. The potential advantages of this approach, relative to treatment with 5-FU, include increased efficiency in the conversion of fluorinated pyrimidine into the fully-activated form (FdUMP), and fewer required steps of enzymatic activation.

5-Fluoro-2'-deoxyuridine (Sigma) was converted to 5'-*O*-(4,4'-Dimethoxytrityl)-5-Fluoro-2'-deoxyuridine 3'-(Cyanoethyl *N,N*-Diisopropylphosphoramidite) [FdU-amidite] according to standard procedures. 5'-*O*-(4,4'-Dimethoxytrityl)-5-fluoro-2'-deoxyuridine was attached to controlled pore glass beads (CPG), and the derivatized CPG was subsequently packed into 10 μmol columns. Each column was subjected to nine coupling cycles with the FdU-amidite using the standard coupling cycle for the ABI 380-B DNA synthesizer. The resulting decamers were cleaved and desalted, and purified using polyacrylamide gel electrophoresis (PAGE; 20% gel). A clonogenic assay was used to measure the cytotoxicity of 5-FU and FdUMP[10]. FdUMP[10] was evaluated in the NCI 60 cell line screen and evaluated for *in vivo* tolerance using Balb/c mice injected by i.v. push administration

Exposure of H630 cells to either 5-FU or FdUMP[10] reduces the number of viable cells. The effectiveness of either drug is enhanced by longer exposure times, however, longer exposure times enhance the effectiveness of FdUMP[10] to a greater extent than 5-FU. At long exposure times, FdUMP[10] is considerably more effective than 5-FU at inhibiting the clonogenic survival of H630 cells. The enhancements factor is greater than the 10-fold increase in the molar equivalence of fluorinated pyrimidine in FdUMP[10] compared to 5-FU. The relative efficiency of 5-FU and FdUMP[10] at inhibiting the proliferation of cells that overexpress TS is dramatically different. H630-10 cells (TS overexpression 20-fold) are resistant to 5-FU, but as sensitive to FdUMP[10] as H630 cells. High sensitivity to FdUMP[10] is observed for non-small cell lung cancer, melanoma, ovarian cancer and renal cancer in the NCI 60 cell line screen. FdUMP[10] is well-tolerated *in-vivo*. Doses of 5-FU above 40 mg/kg/dose qdx3 result in significant weight loss and mortality while administration of FdUMP[10] at 200 mg/kg/dose does not induce any mortality.

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