In vitro activity and mechanism of action of a duplex and multidrug of ethynylcytidine and 5-fluorodeoxyuridine

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INTRODUCTION

Drug resistance due to decreased cellular uptake by transporters and deficiency in key activating enzymes are limitations of many standard anticancer therapies with nucleoside analogs. Both can be circumvented by drug complexes, which when linked via a lipid chain may also bypass transporter systems. The multi- and duplex drugs in this study were composed of two different nucleoside analogs. In the multidrug ETC-L-5FdU, the mononucleotide of 5-fluoro-deoxyuridine (5FdU) was coupled via a glycerophospholipid-linkage to the mononucleotide of ethynylcytidine (ETC). In the duplex ETC-5FdU, the mononucleotide of 5FdU was coupled via a phosphodiester to the mononucleotide of ETC. When enzymatically cleaved behind the phosphate group the nucleotides (FdUMP, ETCMP) will be released. In this way thymidine kinase (TK) and uridine/cytidine kinase (UCK) required for monophosphorylation may be redundant. FdUMP irreversibly inhibits thymidylate synthase (TS), which is one of the most important actions of 5FdU. The aim of the study was to determine the level of cytotoxicity, whether the drugs are cleaved in- or outside the cell and whether the drugs are cleaved into a nucleoside or nucleotide. Moreover we used a liposomal formulation of ETC-L-5FdU to determine whether this would improve its activity and/or cleavage

RESULTS AND DISCUSSION

Drug effects/cleavage with standard radioactivity assays, HPLC and LC-MS/MS were studied in FM3A/0 mammary cancer cells and its 5FdU resistant variant FM3A/TK. The duplex drug ETC-5FdU was active (IC50 of 2.2 and 79 nM) in FM3A/0 and TK-, respectively. The multidrug ETC-L-5FdU was less active (IC50: 7 nM in FM3A/0 vs 4500 nM in FM3A/TK). Although the liposomal formulation was less active than ETC-L-5FdU in FM3A/0 (IC50:19.3 nM), resistance due to TK deficiency was greatly reduced (IC50 of 75 nM). The prodrugs inhibited TS in FM3A/0 cells, but to a lower extent in FM3A/TK-. In the latter the inhibition potential was liposome > duplex > multidrug. No total phosphorylated 5FdU or FdUMP was detected inside FM3A/TK cells. Inhibition of the transporters and nucleotidases/phosphatases resulted in a reduction of cytotoxicity of the duplex drug, indicating that this drug is cleaved outside the cells to the monophosphates, which was indeed verified by the presence of 5FdU and ETC outside FM3A cells. The multidrug and liposomal formulation were not affected by transporter inhibition and nucleotidase/phosphatase inhibition, indicating circumvention of the transporters.

CONCLUSION

These formulations seem to be effective when a lipophilic linker is used combined with a liposomal formulation, however drug uptake and cleavage still need to be improved for further development.

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