Investigations of modulation of 5-fluorouracil (FU) pharmacokinetics by 5-ethyl-2'deoxyuridine (EUdR) in tumor-bearing mice and rats

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Introduction

The anticancer drug 5-fluorouracil (FU) has been in clinical use since the 1960's. It is used predominantly to treat solid tumors in combination with leucovorin to maximize inhibition of the target enzyme thymidylate synthase (TS). FU is a prodrug, for which anabolism to cytotoxic fluoronucleotides (FNuct) by the tumor is a prerequisite. However, the competing deactivation of the drug by catabolism in liver, peripheral mononuclear cells and sometimes, the target tumor, has caused much interest in the development of inhibitors of the primary catabolic step catalyzed by dihydrodipyrimidine dehydrogenase (DPD)[1]. A metabolite of EUdR, ethyluracil, is considered a competitive inhibitor of DPD, which converts FU to dihydrofluorouracil (H_2FU). This study employs a variety of pharmacokinetic techniques to investigate the effects of EUdR on FU *in vivo* to determine if it can cause increased tumor concentrations of FU and FNuct.

Methods

EUdR was injected i.p at 400 mg/kg, 60 min prior to FU (100 mg/kg iv bolus) into Balb-C mice bearing colon26 tumors, or into Buffalo rats bearing H9168A hepatomas. In 4 mice ± EUdR, plasma samples were taken at regular intervals from 5-300 min post FU injection and were analyzed by reverse-phase HPLC to determine FU and H_2FU levels. In other mice, tumors were excised 2-96 hr post FU ± EUdR, extracted, and analyzed by GC-MS to determine tumor FU concentrations. Uptake and metabolism of FU to FNuct or fluorocatabolites (FCat) in rat hepatomas was studied non-invasively by ^{19}F-MRS from 0-110 min post FU injection as previously described [2]. Rat hepatoma and mouse plasma data were analyzed kinetically to determine maximum drug/metabolite concentrations (Cmax) the area under the concentration time-curve (AUC), the half-life for elimination ($t_{1/2}$), the clearance and the mean-residence time in the body (MRT). Enzyme activites of TS, TP (thymidine phophorylase), TK (thymidine kinase) and DPD were determined by radio-enzymological methods in excised mouse tissues.

Results

Both EUdR and ethyluracil (EUra) decreased DPD activity in tumor by 27%, 50% and in liver by 50%, 80% respectively. After 6-8 hr, EUdR also decreased tumor TP activity by 35%, and *potentiated* FU inhibition of TK and TS: increasing inhibition from 42-93% and 40-63% respectively.

In mouse plasma, the FU Cmax, Tmax, $t_{1/2}\alpha$, AUC or clearance were not altered by EUdR, although the H_2FU Cmax was decreased 2-fold ($p<0.01$). However, the MRT of FU and H_2FU increased by 80% ($p\leq0.05$), and the FU $t_{1/2}\beta$ by 50%, although the latter did not reach significance. Consistent with these findings, EUdR raised the FU concentration by ≥50% in mouse tumors from 6 hr ($p<0.05$).

^{19}F-MRS monitoring *in situ* of FU uptake and metabolism by rat hepatoma, showed that the FU $t_{1/2}$ in the tumor was increased by 70% ($p=0.04$) and the FU Cmax and AUC were raised by a similar amount ($p=0.11$, 0.05) (Figure 1). Although the FNuct/FU ratio was unaltered, the FNuct AUC was increased 3-fold ($p=0.01$). Total FCat was unchanged, while the FCat/FU ratio decreased 2-fold ($p=0.01$).

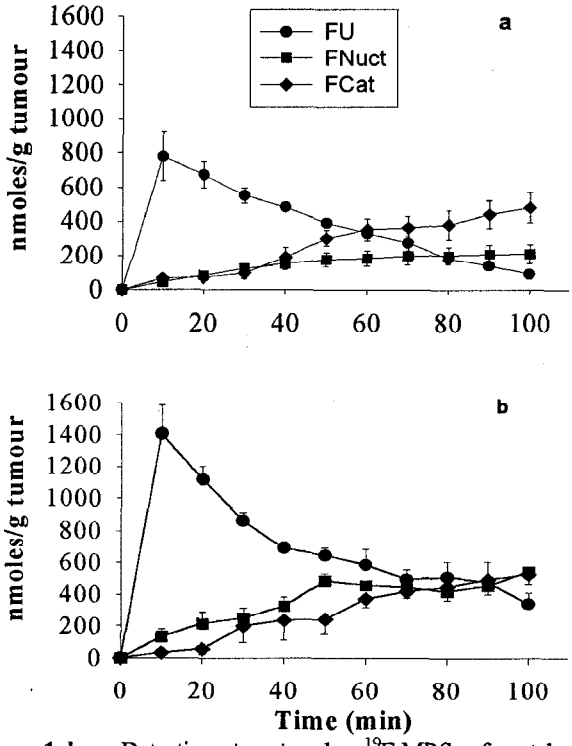

Figure 1ab. Detection *in vivo* by ^{19}F-MRS of uptake and metabolism of FU by rat hepatomas (a) FU alone (b) EUdR + FU.

Summary & Conclusions

The results are consistent with the proposed mechanism of action of the EUdR metabolite, EUra, to inhibit DPD-mediated catabolism of FU to H_2FU. Although mouse plasma FU levels and half-life were not significantly changed, levels of H_2FU were decreased. Mouse colon26 tumor concentrations of FU were significantly increased perhaps because of a significant increase in the average time for *all* FU molecules to reside in the body i.e. MRT. Non-invasive monitoring by ^{19}F-MRS of rat hepatomas confirmed increased uptake and retention of FU which led to a 3-fold increase in tumor FNuct levels.

Rapid inactivation of FU by DPD is a major mechanism of clinical resistance to FU [1]. While EUra is considerably less potent than the DPD inhibitor, 776C85, currently undergoing clinical trial, the high levels of plasma (ethyl)uracil may provide protection against one of the major toxicities of FU, namely myelosuppression [3]. Furthermore, the prolonged inhibition of tumor DPD, TK and TP by EUdR would decrease catabolism of FU and FNuct respectively, leading to increased retention of FU in the tumor.

References

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