In vivo and in vitro MR Biomarkers for Choline Kinase Inhibition in Human Colon Cancer HCT-116

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Introduction
The combined targeted therapy of irinotecan, a topoisomerase I inhibitor, and the phosphokinase inhibitor flavopiridol has shown promise in treating human colon cancer (1, 2). With irinotecan alone, cancer cell cycle is arrested, however without significant cell death. In this study we have investigated in vivo and in vitro MR biomarkers to better understand and monitor HCT-116 cellular responses to the sequential irinotecan or SN38, an active metabolite of irinotecan, and flavopiridol treatment. There were evidences of synergistic effects of the therapy compared to single agent treatment particularly for p53 wild-type HCT-116 cancer cells (1). The beneficiary effect of the combined treatment has also been observed in the MR measurements in the study here.

Methods
In vivo 31P MRSI was performed on a 7T Bruker scanner (Bruker Biospin MRI, Ettlingen, Germany) on HCT-116 xenograft in athymic nude mice. A cohort of 7 mice was treated with irinotecan/CPT11 (100 mg/kg, i.p.) followed by flavopiridol (3 mg/kg, i.p.) 7 hours later. Four control mice were treated with equal volume of saline. 1H decoupled 1D 31P CSI was acquired at day 1, 2, and 7 after the treatment. For the 1D CSI acquisitions 4 mm slice thickness, 45° flip angle, 64 averages, TR of 1.8 s, 8 phase encodes were used. MLEV-16 broadband proton decoupling sequence was used. CK activity assay was carried out on a high-resolution 600 MHz Bruker spectrometer at 20°C. Water suppressed 1D 1H spectra were obtained with 4 averages every 100 seconds for 20 minutes. The enzymatic reaction was carried out in a 10 mM tris buffer solution containing 13 mM β-mercaptoethanol, 1 mM EDTA and 15 mM MnCl2 at pH 7.2. A total of 0.2 units/ml CK from Saccharomyces cerevisiae was used for each experiment. For the time course experiments, HCT-116 cells were treated with SN-38 for 24 hours followed by flavopiridol for 6, 12, 18 and 24 hours, before being trypsinized and collected for NMR or quantitative fluorescence spectroscopy for apoptosis analysis.

In Vitro HR-MAS NMR analysis of HCT-116 cells was performed with a dual 1H/13C 600 MHz MAS probe. The cells were treated with (a) SN-38 for 24 hours followed by flavopiridol for 24 hours; (b) SN-38 for 24 hours; (c) flavopiridol for 24 hours; (d) drug free medium for 24 hours.

Results
Following the combination treatment, a significant drop in phosphocholine(PC) (p= 0.0004) and inorganic phosphate(Pi) (p = 0.0103) levels were observed in HCT-116 xenografts (Figure 1). The treatment of SN-38 and flavopiridol resulted in a gradual decrease of PC/cho ratios (Figure 2). PC/cho ratio decreased by 67% compared to the untreated, by 44% compared to flavopiridol alone, and by 77% compared to SN-38 alone. A strong correlation between apoptosis and cholesterol to CH3 ratios with increasing exposure to flavopiridol is found (r² = 0.9843, p = 0.0008). There was no significant amount of cholesterol with SN-38 treatment alone, corresponding to only 1% apoptosis. Flavopiridol treatment for 24 hours resulted in 5% apoptosis with MR detectable cholesterol/CH3 ratio of 2.33 ± 0.40. The combination treatment resulted in 39% apoptosis and a 2.6-fold increase in the cholesterol/CH3 ratio over flavopiridol alone (p < 0.04) (Figure 3).

Discussion and Conclusion
Irinotecan and flavopiridol can inhibit a wide range of phosphokinases as well as tyrosine and serine kinases. Its inhibitory effects on choline kinase were successfully measured here both in vitro and in vivo by 1H and 31P MR spectroscopy in HCT-116 human colon cancer. In vitro MASS NMR spectroscopy of HCT-116 cells also revealed the apoptosis induced by the treatment correlates very strongly with measured cholesterol levels (3). Our study demonstrated several sensitive MR biomarkers that can be used to measure cellular responses to the combined irinotecan and flavopiridol treatment.

References