In vivo measurement of capecitabine metabolism in human liver by 19-Fluorine Magnetic Resonance Spectroscopy

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Synopsis: In advanced colorectal cancer oral capecitabine is used as an alternative to intravenous 5-fluorouracil (5FU). The last step of capecitabine metabolism into 5FU is the conversion of 5'deoxy-5-fluorouridine (5'DFUR) by thymidine phosphorylase (TP). The rate of 5'DFUR conversion has been related to the level of TP in tumors, which was correlated with tumor response. 5FU catabolites have been associated with 5FU systemic toxicity. Here we demonstrate for the first time that potentially clinically relevant metabolism of capecitabine can be monitored in vivo by 19-fluorine magnetic resonance spectroscopy (^{19}F MRS) in the liver of patients with metastatic colorectal cancer.

Introduction: Oral capecitabine is used as an alternative to intravenous 5-fluorouracil (5FU) in advanced colorectal cancer. The liver is the primary site of capecitabine metabolism as well as the predominant site for metastasis of colorectal cancer. Conversion of 5'deoxy-5-fluorouridine (5'DFUR) by the enzyme thymidine phosphorylase (TP) is the last step of capecitabine metabolism into 5FU. The rate of 5'DFUR conversion has been related to the level of TP in tumors, which was correlated with tumor response *(1;2)*. 5FU catabolites have been associated with 5FU related systemic toxicity. In this study we performed 19-fluorine magnetic resonance spectroscopy (^{19}F MRS) of the liver of patients with metastatic colorectal cancer to monitor capecitabine metabolism.

Patients and methods: Two patients with advanced colorectal cancer treated with oral capecitabine twice daily during two weeks (total dose per day 2500 mg/m² or 2000 mg/m² when combined with intravenous administration of irinotecan (250 mg/m²) on day 1) gave written informed consent before participation. Experiments were approved by the local ethical committee. Measurements were performed on a clinical 1.5 Tesla Siemens MR scanner one hour after oral intake of capecitabine on day 9 or 10 of the two-week period of oral capecitabine intake. A flexible 16 cm ^{19}F MR coil enabled optimal positioning across the liver region. For the first patient with pin-point liver metastasis a pulse-acquire sequence with a 200 μs rectangular RF pulse and a repetition time of 470 ms was used. In the second patient with large livermetastases (>3 cm) pulse-acquire measurements were interleaved with localized ^{19}F MRS using chemical shift imaging (CSI) to differentiate between metabolism in tumor and normal liver tissue. Spherical k-space sampling with Hamming weighted averaging and a repetition time of 450ms was used to obtain optimum SNR. The time resolution was 4 minutes for both pulse-acquire and CSI measurements. To confirm peak assignments of the capecitabine metabolites 5'deoxy-5-fluorocytidine (5'DFCR) and 5'DFUR referenced to 5FU, urine of a patient collected from 3-10 h after capecitabine intake was measured using the same MR protocol, after which 5'DFCR, 5'DFUR (Roche, Mijdrecht, the Netherlands) and 5FU (Teva Pharma, Mijdrecht, the Netherlands) were added consecutively. The dependence of spectral peak position on pH was checked by adding HCl and NaOH to solutions of 5'DFCR with 5FU and 5'DFUR with 5FU in normal saline (0.9%), measuring pH by an EcoScan pH meter (Eutec, Amsterdam, the Netherlands) and peak position by the aforementioned MR protocol.

Results and Discussion: Distinct resonances for capecitabine and its metabolic products 5'deoxy-5-fluorocytidine (5'DFCR) and 5'DFUR levels are observed in the unlocalised spectra (fig. 1). Peak assignments of 5'DFCR and 5'DFUR are confirmed in urine samples (pH 5.87) at 4.0 and 3.4 ppm, respectively. The spectral peak position of 5'DFUR appeared to be pH dependent, with upfield shifting at lower pH. Data fitted to the Henderson Hasselbalch equation show a dissociation constant pK_a of 7.41 (95% confidence interval 7.298-7.517; $R^2 = 0.995$). This pK_a falls within a physiological range and may therefore be used as an in vivo marker of pH. Since the uptake of 5FU in tumor cells is pH dependent, this may be of clinical value. Due to its conversion the concentration of capecitabine in the liver declines to below MR detectable levels within 80 minutes after intake (fig.1). 5'DFCR and 5'DFUR also decline, however, at a slower rate. This agrees with pharmacokinetic parameters in plasma, showing a longer $t_{1/2}$ for 5'DFCR and 5'DFUR compared with capecitabine *(3)*. If the aforementioned preclinical results of correlation between rate of 5'DFUR conversion and tumor response can be confirmed in the clinical setting, ^{19}F MRS can be used as a non-invasive method for predicting response to capecitabine therapy. Localized spectra (fig. 2) show a clear capecitabine resonance from tumor voxels (fig. 2a) compared with normal liver tissue (fig. 2b). In normal liver tissue capecitabine is readily converted by carboxylesterase and therefore in localized spectra its resonance may remain below noise level. In both unlocalized (fig. 1) and localized spectra α-fluoro-β-alanine (FBAL) (fig. 2) can be detected as one of the 5FU catabolites. A large peak 2-2.5 ppm downfield from FBAL is present, usually assigned to 5-fluoro-ureido-propionic acid FUPA *(4)*. Recently, a contribution from FBAL-bile acid conjugate has been described *(5)*. The amplitude of the peak in localized spectra from gallbladder voxels (fig. 2c) reinforces this latter assignment. FBAL-bile acid conjugates would be able to undergo entero-hepatic recirculation and protracted venous infusion of 5FU (PVI 5FU) may establish a higher pool of recirculating catabolite compared with bolus infusion of 5FU, resulting in higher biliary catabolite levels. The mimicking of conventional PVI 5FU is one of the claimed properties of oral fluoropyrimidines. Since an association has been shown between hepatic catabolite levels in patients receiving PVI 5FU and treatment toxicity *(6)* the study of FBAL kinetics may provide insight into capecitabine related toxicity.

Conclusions: Both CSI and pulse acquire ^{19}F MRS of capecitabine metabolism can be performed in vivo in human liver. Therefore, ^{19}F MRS is a potentially important tool for the prediction of response and toxicity in capecitabine treatment.

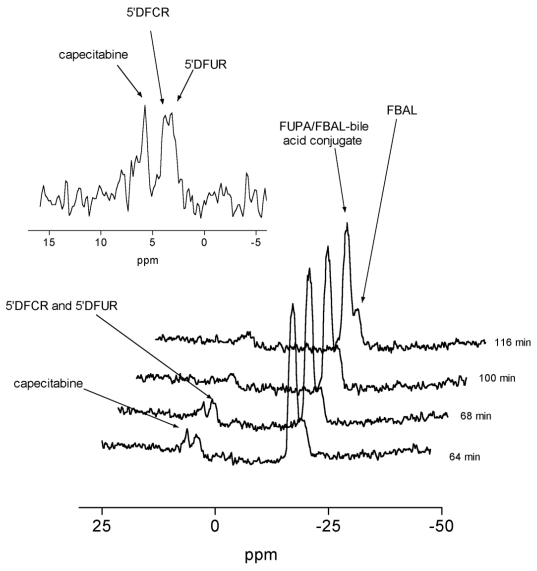

Fig. 1 Spectra obtained by ^{19}F MRS of the liver of patient 1, starting 60 minutes after oral capecitabine intake, using a pulse acquire sequence. Inset: Expanded spectrum of the region from –5 to 15 ppm, showing the average of spectra taken from 60 till 76 minutes after capecitabine intake. 5'DFCR = 5'deoxy-5-fluorocytidine; 5'DFUR = 5'deoxy-5-fluorouridine; FUPA = 5-fluoro-ureido-propionic acid; FBAL = α-fluoro-β-alanine

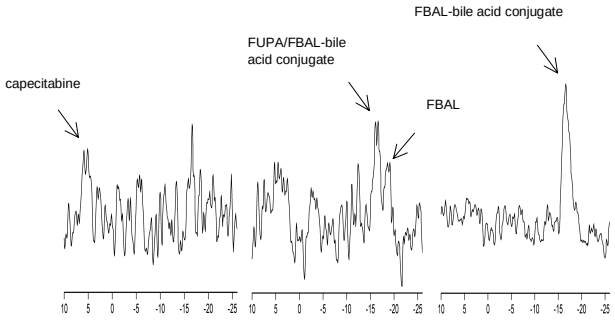

Fig. 2 CSI ^{19}F MRS spectra of patient 2, 60 minutes after oral capecitabine intake. Fig. 2A and 2B: spectra from five tumor voxels and four liver voxels, respectively. Fig. 2C: spectrum from four gallbladder voxels. Abbreviations as in fig. 1

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