

In Vivo Monitoring of Capecitabine Metabolism in Human Liver by ¹⁹F Fluorine Magnetic Resonance Spectroscopy at 1.5 and 3 Tesla Field Strength

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Abstract

In metastatic colorectal cancer the oral 5-fluorouracil (5FU) prodrug capecitabine is used with increasing frequency as an alternative to i.v. 5FU administration. The rate of conversion of capecitabine into 5'-deoxy-5-fluorouridine has been related to tumor response, and 5FU catabolites have been associated with 5FU-related systemic toxicity. Here we demonstrate for the first time that capecitabine, its metabolites 5'-deoxy-5-fluorocytidine and 5'-deoxy-5-fluorouridine, and its catabolites 5-fluoro-ureido-propionic acid, α -fluoro- β -alanine, and α -fluoro- β -alanine-bile acid conjugate can be monitored *in vivo* by ¹⁹F fluorine magnetic resonance spectroscopy (¹⁹F MRS) in the liver of patients with metastatic colorectal cancer. Moreover, we demonstrate an improved signal-to-noise ratio and spectral resolution of the ¹⁹F MRS spectra when measurements are performed at 3 T field strength as compared with measurements at the common clinical field strength of 1.5 T. We conclude that assessment of capecitabine metabolism in patients by ¹⁹F MRS is a promising noninvasive tool for the prediction of its efficacy and toxicity, especially at the now currently available clinical field strength of 3 T.

Introduction

Predicting the sensitivity of a tumor to chemotherapy in cancer patients would enable individualization of therapy, by which unnecessary toxicity in nonresponding patients could be avoided. A correlation between response to therapy and pharmacokinetic parameters measured with ¹⁹F MRS¹ has been shown for the widely used cytotoxic drug 5FU (1). Because ¹⁹F MRS is a noninvasive technique that allows the measurement of 5FU metabolism at specific sites of interest, it is an attractive tool for the prediction of therapy outcome.

In patients with advanced colorectal cancer, oral capecitabine is used with increasing frequency as an alternative to i.v. 5FU treatment, due to its comparable efficacy, ease of administration, and more favorable toxicity profile (2, 3). Capecitabine is preferentially metabolized to 5FU in tumors and liver by a three-step enzymatic process (4), involving conversion into 5'DFCR, followed by conversion into 5'DFUR. In the third step 5'DFUR is metabolized into 5FU by the enzyme TP. In preclinical studies the rate of 5'DFUR conversion as determined by ¹⁹F MRS has been related to the level of TP in tumors (5), and a strong correlation between TP levels in tumors and tumor response has been observed (6). 5FU is additionally metabolized via different biochemical pathways to cytotoxic metabolites. 5FU catabolites like FBAL have been associated with 5FU-related systemic

toxicity (7). Here we report for the first time that the metabolism of capecitabine after oral intake can be monitored *in vivo* by ¹⁹F MRS.

Until now human ¹⁹F MRS studies have been performed at standard clinical MR systems operating at 1.5 T field strength. 3 T MR scanners have become available recently for clinical application. For ¹⁹F MRS the use of higher field strengths is expected to result in an increased SNR and an improvement of spectral resolution, which would better facilitate its use in clinical examinations of fluorinated drugs. Therefore, in this study we have included a first investigation in patients of ¹⁹F MRS at 3 T.

Materials and Methods

Because the liver is the primary site of capecitabine metabolism as well as the predominant site for metastasis of colorectal cancer, we performed nine ¹⁹F MRS measurements in the liver of five patients with advanced colorectal cancer (Table 1). Patients were treated with oral capecitabine during 2 weeks. All of the patients had a Karnofsky performance status $\geq 90\%$ and a normal liver function. ¹⁹F MRS measurements were performed during at least 40 min, starting ~ 1 h after oral intake of capecitabine in the 2-week period of oral capecitabine intake. Patients gave written informed consent, and the experiments were approved by the local ethical committee.

¹⁹F MRS measurements were performed on both a clinical 1.5 T and a 3 T whole body Siemens MR system. A flexible 16-cm ¹⁹F MR coil was used to enable optimal positioning across the liver region to receive the MR signals of capecitabine and its metabolites from that region. For all of the measurements a pulse-acquire sequence was used with a repetition time of 470 ms. In patients 2, 3, and 4 (Table 1) pulse-acquire measurements were interleaved with localized measurements by ¹⁹F MRSI (8). The use of MRSI gives the opportunity to differentiate between the conversion of capecitabine in tumor and normal liver tissue. An $8 \times 8 \times 8$ MRSI was used with a voxel size of $4 \times 4 \times 4$ cm. Both pulse-acquire and MRSI measurements were optimized for signal to noise (9) with a temporal resolution of 4 min. No respiratory gating was used. Resonances in the ¹⁹F MR spectra were analyzed using MRUI software.² Percentage changes during the measurement interval in ¹⁹F MR spectral peak areas obtained by the pulse acquire sequence were determined for DFCR+DFUR and the 5FU catabolites. For DFCR+DFUR the difference in MR peak area of the last spectrum that showed the MR peak of DFCR+DFUR and the peak area of the first spectrum that showed this MR peak was normalized to the peak area of the first spectrum. For 5FU catabolites the same method was applied.

To confirm peak assignments of the capecitabine metabolites 5'DFCR and 5'DFUR referenced to the spectral position of the 5FU signal, urine of patient 2 collected from 3–10 h after capecitabine intake was measured at 1.5 T 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 examined by adding HCl and NaOH to solutions of 5FU with 5'DFCR or 5'DFUR in normal saline (0.9%), measuring pH by an EcoScan pH meter (Eutec, Amsterdam, the Netherlands) and peak position by the aforementioned MR protocol.

To quantify the improvement of SNR at 3 T in comparison with 1.5 T we determined the amplitude of the catabolite peaks in the ¹⁹F MR spectra of patients 2, 3, and 4 obtained at a similar time point after capecitabine intake

Received 1/25/03; revised 9/18/03; accepted 10/7/03.

Grant support: Dutch Cancer Society, Grant KUN 2000-2307

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¹ The abbreviations used are: ¹⁹F MRS, ¹⁹F fluorine magnetic resonance spectroscopy; DFCR, deoxy-5-fluorocytidine; DFUR, deoxy-5-fluorouridine; FBAL, α -fluoro- β -alanine; 5FU, 5-fluorouracil; MRSI, magnetic resonance spectroscopic imaging; PVI, protracted venous infusion; SNR, signal-to-noise ratio; TP, thymidine phosphorylase; MR, magnetic resonance.

² Internet address: <http://www.mrui.uab.es/mrui/mruiHomePage.html>.

Table 1 Patient characteristics and percentage change of DFUR+DFCR and 5FU catabolites during the measurement interval (for details: see Materials and Methods). The time period during which MR peaks were visible in the measurement interval is indicated (in minutes after capecitabine intake). For the 5FU catabolites this time period corresponded in each patient to the complete measurement interval.

| Patient | Sex | Age (years) | Site of metastases | MR field strength | Treatment | % Change of DFUR + DFCR during measurement interval | % Change of 5FU catabolites during measurement interval | Follow-up |
|---------|-----|-------------|--------------------|-------------------|---|---|---|--|
| 1 | M | 60 | Liver | 1.5T | capecitabine 1000 mg/m ² , twice daily, day 1–14 irinotecan 250 mg/m ² , day 1 cycle 2, day 9 | –59% (59–105 min) | –19% (59–113 min) | Partial response after 3 cycles |
| 2 | M | 57 | Liver | 1.5T | capecitabine 1250 mg/m ² twice daily, day 1–14 cycle 1, day 10 | –59% (76–119 min) | +17% (76–119 min) | Stable disease after 3 cycles, partial response after 6 cycles, continued partial response after 15 cycles |
| | | | | 3.0T | cycle 16, day 9 | –100% (51–54 min) | +5% (51–75 min) | |
| | | | | 1.5T | cycle 16, day 10 | no DFUR + DFCR (42–63 min) | –1% in 63 min (42–63 min) | |
| 3 | M | 72 | Lung | 3.0T | capecitabine 1250 mg/m ² twice daily, day 1–14 cycle 5, day 11 | –39% (60–84 min) | +64% (60–84 min) | Partial response after 3 cycles |
| | | | | 1.5T | cycle 5, day 12 | –40% (63–85 min) | +19% (63–85 min) | |
| 4 | M | 55 | Liver | 1.5T | capecitabine 1250 mg/m ² twice daily, day 1–14 | | | Continued partial response after 6 cycles |
| | | | | 3.0T | cycle 7, day 2 | +334% (35–75 min) | +592% (35–75 min) | |
| 5 | F | 70 | Lung | 1.5T | capecitabine 1250 mg/m ² twice daily, day 1–14 cycle 3, day 11 | +48% in 81 min (45–81 min) | –5% in 89 min (45–89 min) | Stable disease after 3 cycles |
| | | | | 1.5T | cycle 3, day 11 | +130% in 79 min (41–79 min) | +64% in 84 min (41–84 min) | |

from the 1.5 T and 3 T measurements. The amplitude was divided by the SD of the noise from the same spectrum. This ratio acquired for the measurement at 3 T was divided by the ratio for that at 1.5 T to obtain the factor of improvement in SNR for each patient.

Results and Discussion

¹⁹F MR spectra taken from the liver showed distinct resonances for capecitabine and its metabolic products (Fig. 1). Spectra obtained at 3 T showed a factor 1.3–3 higher SNR and an improved spectral resolution in comparison with those obtained at 1.5 T (Fig. 2), as may be expected from measurements at higher field strengths.

We confirmed peak assignments to 5'DFCR and 5'DFUR in measurements of urine samples (pH 5.87) at 4.0 and 3.4 ppm, respectively. The spectral peak position of 5'DFUR showed pH dependence, with upfield shifting (more toward 5FU) at lower pH. A similar pH-dependent upfield shifting has been described for various other 5FU anabolites (10). The data were fitted to the Henderson Hasselbalch equation $pH = pK_a + \log[(x - a)/(b - x)]$ with x representing the spectral peak position in ppm. A dissociation constant pK_a of 7.41 was found, with $a = 3.35$ and $b = 5.53$ ($R^2 = 0.995$). Because the pK_a of 5'DFUR falls within the physiological pH range and as this range corresponds with a relatively large shift in ppm values, 5'DFUR can be used as an *in vivo* marker of tissue pH. From our spectra the pH of liver tissue was found to be 7.39, which agrees with *in vivo* pH values in liver tissue measured by ³¹P MRS (11). In isolated tumor cells intra:extracellular 5FU ratio correlated with extracellular pH, intracellular pH, and the pH gradient across the cell membrane (12). In an animal model a decrease in local tissue pH from 7.3 to 6.9 was associated with a 2.5-times increase of the $t_{1/2}$ of 5FU, indicating a trapping of 5FU in the tumor (13). Because of the relation between trapping of 5FU in the tumor and tumor response (1), *in vivo* measurement of pH by the 5'DFUR signal shift can be useful in the prediction of therapy outcome.

The time course of capecitabine metabolism is shown in Fig. 1 for

patient 1. In this patient the concentration of capecitabine in the liver declined to below MR detectable levels within 80 min after intake due to its conversion and clearance (Fig. 1). The conversion and clearance

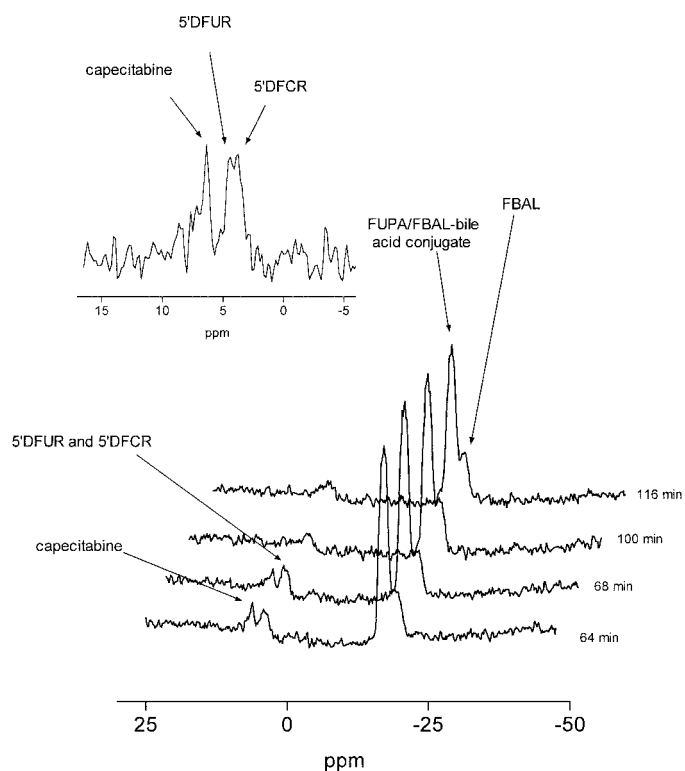


Fig. 1. Sequential ¹⁹F MR spectra of the liver of patient 1, obtained by a pulse acquire sequence at 1.5 T and starting 60 min after oral capecitabine intake. Inset, expanded spectrum of the region from –5 to 15 ppm, showing the average of spectra taken from 60 to 76 min after capecitabine intake.

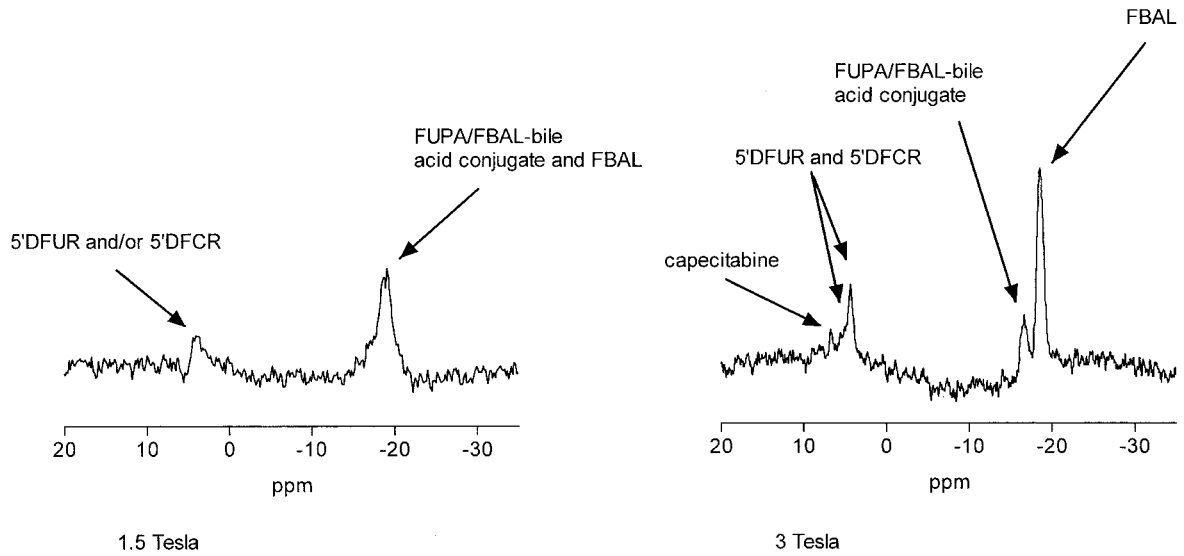


Fig. 2. ¹⁹F MR spectra of the liver of patient 4, obtained by a pulse acquire sequence at 1.5 T (left) and 3 T (right), showing the average of two spectra taken 68 and 76 min after oral capecitabine intake. To facilitate comparison the 3 T spectrum have been scaled to equal noise levels with the 1.5 T spectrum.

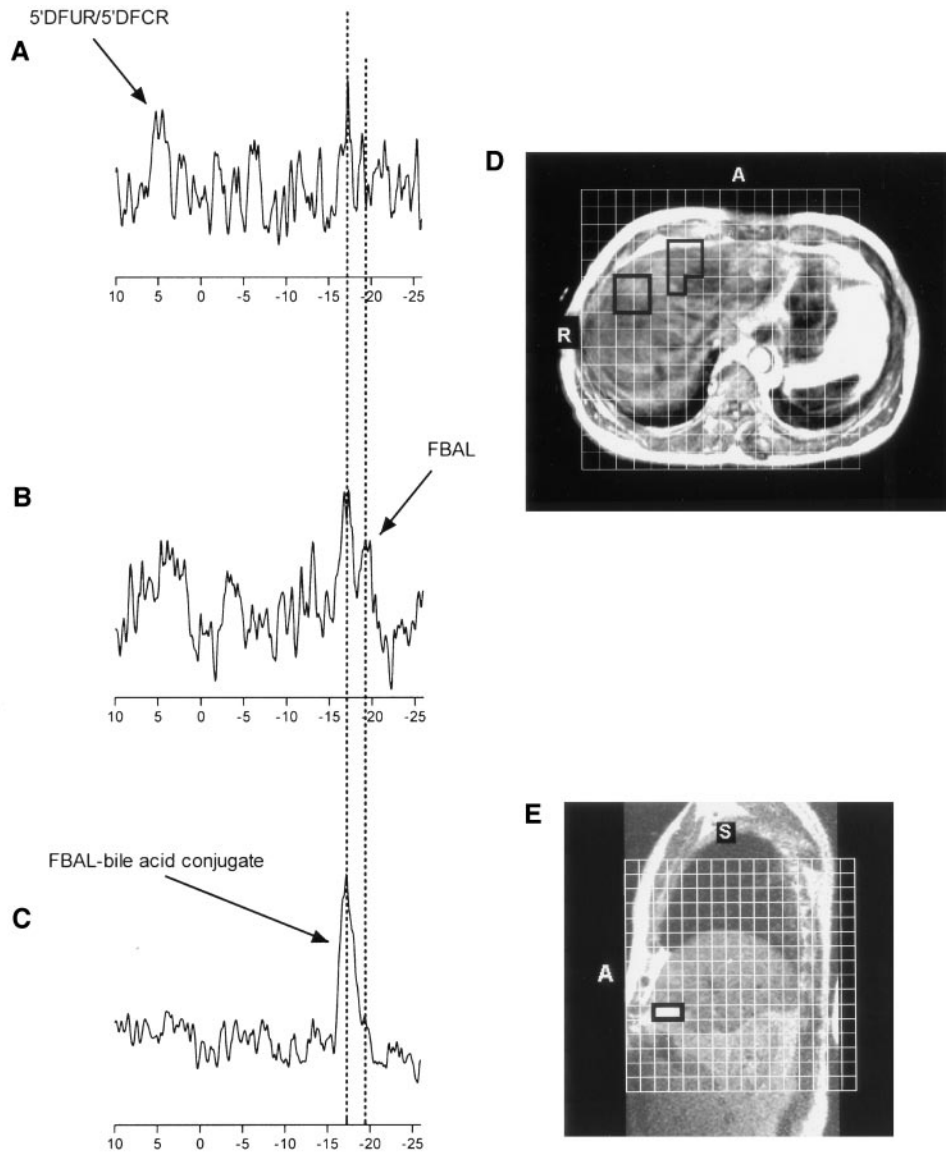


Fig. 3. Localized spectra obtained by ¹⁹F MR spectroscopic imaging at 1.5 T of patient 2, 60 min after oral capecitabine intake on day 10. A and B show the spectra from five tumor voxels and four liver voxels as indicated on the T1 weighted image (D), respectively. The tumor has a maximum diameter of ~7 cm, and its center is located at a distance of at least 8 cm from the coil. C shows the spectrum from four gallbladder voxels as indicated on the T2 weighted image (E). A, anterior; r, right; S, superior; other abbreviations as in Fig. 1.

of capecitabine metabolites was highly variable between patients, as is indicated in Table 1. In all of the patients 5'DFCR and 5'DFUR were MR detectable for a prolonged time compared with capecitabine. This is in accordance with pharmacokinetic parameters in plasma, which showed a longer $t_{1/2}$ for 5'DFCR and 5'DFUR compared with capecitabine (14). In fasting patients a delay in occurrence of maximum plasma concentration and area under the plasma concentration curve has been described for capecitabine and its metabolites, with no effect on the apparent elimination half-life (15). In all of our patients capecitabine was administered in nonfasting condition, as has been the procedure in clinical trials.

5FU concentration was below MR detectable levels due to its rapid metabolic conversion. FBAL, a 5FU catabolite, was detected in both unlocalized (Fig. 1) and localized spectra (Fig. 3). Others have shown in humans that in urine FBAL was the major metabolite of capecitabine (16). The large peak in our spectra 2–2.5 ppm downfield from FBAL is usually assigned to the 5FU catabolite 5-fluoro-ureido-propionic acid (17). At this spectral position a contribution from FBAL-bile acid conjugate has been described recently (18). Evidence from both ¹⁹F nuclear MR spectroscopic studies (19) and high-performance liquid chromatography (20) indicate that the major biliary metabolites of 5FU are conjugates of FBAL. The amplitude of the peak in localized spectra from gallbladder voxels (Fig. 3C) supports this latter assignment. FBAL-bile acid conjugates would be able to undergo enterohepatic recirculation. PVI of 5FU may establish a higher pool of recirculating catabolite compared with bolus infusion of 5FU, resulting in higher biliary catabolite levels (18). The mimicking of conventional PVI of 5FU is one of the claimed properties of oral fluoropyrimidines. Because hepatic catabolite levels are correlated with toxicity in patients receiving PVI of 5FU (21), FBAL kinetics measured by ¹⁹F MRS may be an early predictor of capecitabine-related toxicity.

If the correlation between the rate of 5'DFUR conversion and tumor response (5, 6) is confirmed in the clinical setting, ¹⁹F MRS can also be used as a noninvasive method for predicting the antitumor response of capecitabine. This would be highly relevant, because advanced colorectal cancer is a frequently occurring cancer, and only a subset of patients responds to fluoropyrimidine-based therapy. We conclude that ¹⁹F MRS of capecitabine metabolism or of other orally administered prodrugs (22) is a promising tool for the individualization of chemotherapy, especially when higher field strengths can be applied for better sensitivity.

Acknowledgments

We thank Martin O. Leach and David J. Collins for helpful discussions.

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