^{19}F-magnetic resonance spectroscopy in patients with liver metastases of colorectal cancer treated with 5-fluorouracil

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The purpose of this study was to examine the uptake and metabolism of 5-fluorouracil (5-FU) in human liver metastases. Patients with liver metastases of colorectal cancer were treated with 5-FU (500/600 mg/m^2) + folinic acid with or without trimetrexate. The clinical application of ^{19}F-magnetic resonance spectroscopy (MRS) of 5-FU in a random group of patients (n = 17) was investigated. MR spectra of all patients showed 5-FU and catabolite resonances, and fluoronucleotides were also seen in seven patients. A correlation was found between maximum levels of 5-FU catabolites as measured by ^{19}F-MRS and response in a group with larger metastases. However, such correlation was not observed in a group with smaller metastases, probably because of a significant contribution of normal liver tissue to the MR spectra. *Anti-Cancer Drugs* 15:229–233 © 2004 Lippincott Williams & Wilkins.

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Introduction

5-Fluorouracil (5-FU) is still the most important cytotoxic agent in the treatment of colorectal cancer. It is combined with different modulators in order to enhance effectiveness. There may be three conditions of importance for tumor response to 5-FU [1]: (i) adequate exposure of the tumor to 5-FU, requiring good tissue accessibility, (ii) adequate uptake of 5-FU from the vascular space into the cellular (and interstitial) space of the tumor and (iii) favorable biochemical characteristics of the tumor cell. Magnetic resonance spectroscopy (MRS) is a non-invasive method to measure the second and third conditions, presence and conversion of 5-FU in the tumor [2,3]. In the present study, ^{19}F-MRS was performed to obtain information about 5-FU uptake and metabolism in patients with liver metastases of colorectal cancer. The results were compared with the outcome of the radiological response evaluation.

^{19}F-MRS is a method to investigate the pharmacokinetics of fluorine-containing drugs like 5-FU [2,3] and its conversion into anabolites (fluoronucleotides and nucleosides) and catabolites [α-fluoro-β-alanine (FBAL), α-fluoro-β-ureidopropionic acid (FUPA) and dihydrofluorouracil (DHFU)]. It is assumed that the anabolic processes predominate inside tumor cells, whereas the catabolism takes place in the liver and also in extrahepatic tissue [4–6]. By recirculation, catabolites can enter tumor cells afterwards. Only a few groups have performed ^{19}F-MRS studies of 5-FU in patients [7–12]. These studies showed that ^{19}F-MRS could be a powerful technique to monitor the intratumoral metabolism of 5-FU in patients and may be used to predict therapy outcome.

We performed this study to investigate the clinical application of ^{19}F-MRS of 5-FU in a random group of patients with liver metastases of colorectal cancer, so not only selected for large, superficial tumors in the liver, but also patients with smaller liver metastases were included.

Patients and methods

Patients

Seventeen patients (two female, 15 male) with colorectal cancer with liver metastases and no previous chemotherapy were studied with ^{19}F-MRS. For patient characteristics, see Table 1. All patients had one or more measurable liver metastases of at least 2 cm diameter in the field of the coil (within 8 cm of the skin surface) and were scheduled to receive 5-FU-containing chemotherapy as described below. The mean age of the patients was 59 years (range 33–77 years). Primary tumors were located in the colon (n = 12) or rectum (n = 5). Ten patients showed isolated liver metastases; seven patients had also metastases in lung, lymph nodes, peritoneum or a local recurrence. The study was approved by the local ethical committee and informed consent was obtained from all patients prior to the examinations.

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Table 1 Patient data

No.	Age	Sex	Location of primary tumor	Location of metastases	V_m in FOV (cm^3)	V_m/d^2 (cm)	5-FU/TMTX dose (mg/m^2)	Δ tumor size (%)
3	35	M	rectum	liver	9	0.18	600	68 (SD)
5	77	M	colon	liver	26	0.36	600	100 (SD)
6	53	M	colon	liver	6	0.31	600	30 (PR)
11	60	M	return	liver, lung, local recurrence	0.5	0	300	45 (PR)
13	52	M	colon	liver, peritoneum	8	0.72	600	100 (SD)
15	75	M	rectum	liver, lung	19	0.23	600	143 (PD)
16	75	M	colon	liver	6	0.25	500/110	72 (SD)
17	45	M	colon	liver	4	0.11	450	54 (SD)
1	59	M	colon	liver, local recurrence	74	6.42	500/110	37 (PR)
2	65	M	colon	liver	179	9.69	600	86 (SD)
4	55	M	colon	liver, lymph nodes	72	8.56	500/110	100 (SD)
7	33	M	colon	liver	42	5.34	500	77 (SD)
8	72	M	colon	liver	50	3.88	600	100 (SD)
9	49	F	rectum	liver, lung	92	4.73	600	136 (PD)
10	73	M	colon	liver, lung	34	1.11	600	95 (SD)
12	56	F	colon	liver	24	0.65	450	149 (PD)
14	62	M	rectum	liver	24	0.61	600	205 (PD)

The sequence of patient numbers is comparable with Table 2.

Treatment

Patients were treated with 5-FU 600 mg/m^2 + folinic acid 200 mg/m^2 ($n = 14$) or 5-FU 500 mg/m^2 + folinic acid 200 mg/m^2 + trimetrexate 110 mg/m^2 ($n = 3$). 5-FU was administered in a non-fasting condition as a short i.v. infusion within 5 min, preceded by folinic acid applied in 1 h and sometimes also by trimetrexate applied in 1 h, 24 h before 5-FU. Treatment was given weekly, 6 out of 8 weeks. On three occasions the dose of 5-FU had to be reduced, because of toxicity (Table 1).

Assessment of response

Bidimensional measurement of tumor lesions was performed with conventional computed tomography (CT) or ultrasound before and after each treatment cycle, within 8 weeks after the start of treatment. Radiological responses were classified as complete remission (CR) for total disappearance of the tumor mass, partial remission (PR) for a decrease by at least 50%, stable disease (SD) for a change of tumor size between –50% and + 25%, and progressive disease (PD) for an increase by at least 25% or new lesions. Furthermore, for this study the percentage change of the radiological response (Δ tumor size) after the first cycle of 5-FU therapy was determined.

^{19}F-MRS

^{19}F-MRS was performed on a 1.5-T whole-body MR system. A RF surface coil of 10 cm loop diameter was employed. A sample of trifluoroacetic acid (CF_3COOH) fixed in the centre of the coil was used for calibrating chemical shifts δ (δ 5-FU was set to 0 p.p.m.), and as a ^{19}F-MR signal reference to account for different coil loading and to normalize peak integrals. For ^{19}F-MR data acquisition an initial spectral width of 20 kHz was used to detect the reference signal and for the actual data a spectral width of 3500 Hz was used, zoomed in on the region with relevant signals. Spin excitation was performed with an adiabatic 90° pulse. A repetition time of 256 ms was used, which is somewhat arbitrary and difficult to set precisely at an optimum, as T_1 values of ^{19}F metabolite spins *in vivo* in patients are uncertain (values have been reported between around 0.12 and 1.6 s) and may differ per metabolite [12–14]. Data acquisition took place in blocks of 4 min (938 acquisitions) with a median total duration of 40 min (range 28–52 min) after the bolus injection. Patients were positioned on the surface coil in the table, lying on their stomach or right side. Gradient echo images were recorded to confirm that the sensitive volume of the surface coil covers a significant portion of the liver. The metastases in the liver were located as close as possible to the RF coil. Patients were examined preferably during week 6 of the first treatment cycle

Data analysis

Spectral data were analyzed on a SUN computer, using the software package MRUI [15]. Detected ^{19}F-MR resonances were assigned on the basis of published chemical shift data [9,10]. 5-FU and FBAL resonances were analyzed by means of a fit routine (Lorentzian line). The areas (^{19}F-MR signal intensities) of the fitted lines were integrated and divided by the peak area of the reference CF_3COOH. These normalized peak integrals were plotted as a function of time. The parameters C_{max} and AUC define maximum signal intensity and area under the concentration–time curve, and were determined graphically from the fitted curve. They were corrected for the administered 5-FU dose.

The volume of the examined liver metastases (V_m) within the field of the ^{19}F-MR coil was approximated by an ellipsoidal shape: $V_m = \pi/6\ (d_x \times d_y \times d_z)$, where d_x, d_y and d_z are orthogonal diameters that are measured by means of MRI. The median of these tumor volumes was 24 cm^3

(range 0.5–179 cm^3). The local RF sensitivity of the surface coil is confined to a half-sphere (radius about 5 cm), with decreasing sensitivity at increasing axial distance (d) with respect to the centre of the coil. Two groups of patients were distinguished: (a) $V_m \times ([d/r]^2 + 1)^{-3/2} \leq 12$ (range 0–12) and (b) $V_m \times ([d/r]^2 + 1)^{-3/2} > 12$ (range 19–149), where $([d/r]^2 + 1)^{-3/2}$ is a rough approximation of the axial coil B_1 profile (Biot-Savart). The median value of $V_m \times ([d/r]^2 + 1)^{-3/2}$ of 11 divides the patients in two groups: (a) with a smaller contribution of the metastases to the ^{19}F-MRS signal and (b) with a larger contribution. In group (a), the contribution of the normal liver tissue to the signal is larger than in group (b). In order to determine $t_{1/2}$ of 5-FU, 5-FU peaks were fitted to a mono-exponential equation, $y = A \times e^{-kt}$ [12,16].

Statistics

Data are given in mean + SD and in median values. In Table 2, ^{19}F-MRS parameters were correlated with Δ tumor size (determined bidimensionally by CT or ultrasound) using Spearman correlation analysis. A correlation coefficient > 0.70 was considered to imply a good correlation.

Results

Response to treatment

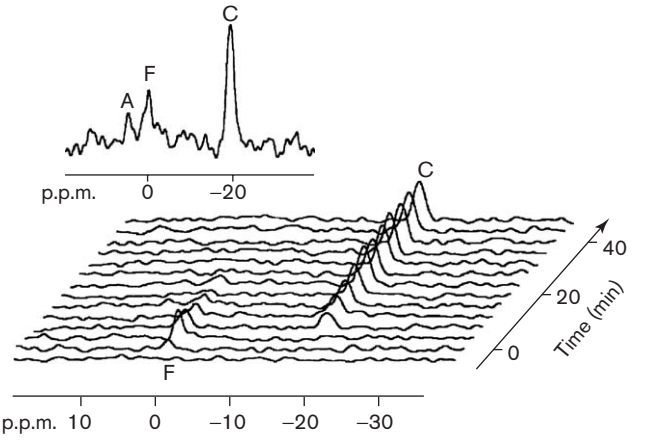
Based on CT (in 14 patients) or ultrasound (in three patients), of the 17 patients studied with ^{19}F-MRS three patients were classified as having a PR, 10 as having SD and four as having PD. The response rate was determined after the first evaluation after 2 months. No CRs were observed at this time.

^{19}F-MRS

^{19}F-MRS data acquisition was started 4 min before 5-FU administration and terminated after the 5-FU signal had disappeared, i.e. after a mean period of 42 ± 7 min (Fig. 1). ^{19}F-MR resonances for 5-FU (within 4–8 min) and FBAL (after 13 ± 5 min) were observed, with a chemical shift difference of 18.9 ± 0.6 p.p.m. between both peaks. FUPA resonates about 1.8 p.p.m. down field from FBAL, but was mostly not observed as a resolved peak. Also sporadically observed was the catabolite DHFU at about –32 p.p.m. Furthermore, in seven out of 17 cases a fluoronucleotide signal could be seen at 4.9 ± 0.5 p.p.m. during 4 min (Fig. 1). An example of time courses of the normalized peak integrals of 5-FU and FBAL is shown in Fig. 2.

In order to reveal differences in patients with metastases of varying sizes, two groups of patients were distinguished, with smaller and large volumes of metastases (see Table 2). The C_{max} of 5-FU and FBAL was determined, as well as the AUC of the 5-FU curve. Furthermore, the $t_{1/2}$ of 5-FU washout was determined by

Fig. 1



^{19}F-MR spectra of the liver with metastases of a 5-FU-treated patient. Shown are sequential spectra at 4-min intervals after 5-FU administration from 0 to 3 min. A, anabolites; C, catabolites; F, 5-FU. Insert: A, F and C at 16–20 min.

^{19}F-MRS. No increased retention of 5-FU was found in responding patients according to the $t_{1/2}$ values.

In the patients with $V_m \times ([d/r]^2 + 1)^{-3/2} \leq 12$ (group a, $n = 10$) with a larger contribution of the normal liver tissue to the ^{19}F-MRS signal a high correlation was found between Δ tumor size and volume of metastases (in the field of the coil) (Spearman correlation coefficient 0.8). In the patients with $V_m \times ([d/r]^2 + 1)^{-3/2} > 12$ (group b, $n = 7$) with a larger contribution of the metastases to the ^{19}F-MRS signal a correlation was found between Δ tumor size and C_{max} of catabolites (Spearman correlation coefficient 0.7). The presence of a weak fluoronucleotide signal was seen in all three patients with PRs, but also in two patients with SDs and even in two with PDs, and this does not correlate with Δ tumor size.

Discussion

Advanced colorectal cancer with liver metastases is a frequently occurring problem and treatment with cytotoxic agents results only in a third of the patients in PR or CR. In another third of the patients stable disease is achieved. It would be useful to have a method to predict in an early phase response to 5-FU-containing therapy.

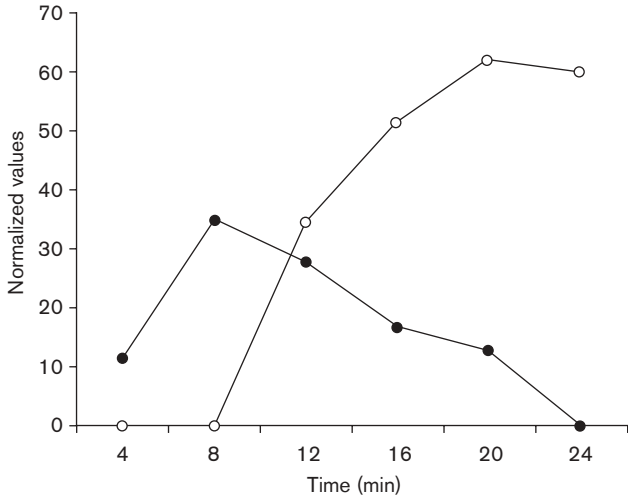
Only a few groups have performed ^{19}F-MRS studies of 5-FU in patients. Wolf and Presant and colleagues were able to detect 5-FU, but none of its metabolites, in several tumors of patients with different types of cancer [3,8]. The half-life ($t_{1/2}$) of 5-FU in tumors was shown to be much longer (20 min or more) compared to the $t_{1/2}$ of 5-FU in blood (8–12 min). The extent of trapping of 5-FU in tumors correlated with the antitumor response. Findlay *et al.* [9] found a correlation between

Table 2 **Patient data and ^{19}F-MRS results**

No.	5-FU dose (mg/m^2)	V_m in FOV (cm^3)	C_{max} 5-FU	AUC 5-FU	$t_{1/2}$ 5-FU (min)	Detection 5-FU (min)	C_{max} catabolites	Δ tumor size (%)
Group (a) $V_m \times ([d/r]^2+1)^{-3/2} < 12$ ($n=10$)								
3	600	9	32	0.29	10	28	85	68 (SD)
5	600	26	92	1.72	12	44	160	100 (SD)
6	600	6	66	1.13	9	40	118	30 (PR)
11	300	0.5	53	0.43	4	12	63	45 (PR)
12	450	24	78	0.88	8	20	184	149 (PD)
13	600	8	77	1.18	5	28	95	100 (SD)
14	600	24	28	0.53	34	32	23	205 (PD)
15	600	19	57	1.10	11	44	98	143 (PD)
16	500	6	46	0.82	7	32	54	72 (SD)
17	450	4	67	0.94	7	32	91	54 (SD)
median	600	8.5[a]	61.5	0.91	8.5	32	93	
Group (b) $V_m \times ([d/r]^2+1)^{-3/2} \geq 12$ ($n=7$)								
1	500	74	38	0.88	8	36	62	37 (PR)
2	600	179	55	0.81	6	28	93	86 (SD)
4	500	72	58	0.87	8	28	90	100 (SD)
7	500	42	68	1.01	10	32	52	77 (SD)
8	600	50	45	0.63	6	28	63	100 (SD)
9	600	92	68	0.99	7	32	120	136 (PD)
10	600	34	65	0.84	9	32	80	95 (SD)
median	600	72	58	0.87	8	32	80[a]	

[a]Spearman correlation coefficients between change in tumor size and ^{19}F-MRS results/patient data ≥ 0.70.

Fig. 2



Time courses of normalized peak integrals of 5-FU and FBAL of the same patient. Solid circles = F_{rel}; open circles = C_{rel}.

a detectable 5-FU signal observed during the first 8 weeks of treatment and response to treatment with continuous low-dose i.v. infusion of 5-FU ± interferon-α. Schlemmer *et al.* [10] found a correlation between enhanced 5-FU levels in liver metastases and response. The same group also performed ^{19}F-MRS on cervical lymph node metastases of patients with head and neck carcinoma [11]. Patients were treated with simultaneous radiochemotherapy containing 5-FU. The levels of 5-FU and its catabolite FBAL were higher at the second chemotherapy cycle compared with the first examination. No correlation between response to treatment and ^{19}F-MRS parameters was found. Li *et al.* [12] obtained localized ^{19}F-MR spectra of the liver of patients receiving 5-FU using three-dimensional chemical shift imaging. These studies showed that ^{19}F-MRS could be a powerful technique to monitor the intratumoral metabolism of 5-FU in patients and may be used to predict therapy outcome.

In the present study, clear signals from 5-FU and FBAL were observed in the spectra of all patients. Sometimes a small peak was seen at the position of the fluoronucleotides. Findlay *et al.* [9] also showed an anabolite signal in the ^{19}F-MR spectra of some patients.

In the group with a larger contribution of normal liver tissue to the ^{19}F-MRS signal (group a) there was a correlation between decrease in tumor size (change determined bidimensionally by CT or ultrasound) and small metastases (in the field of the coil). This is in congruence with clinical findings. We found no correlation between the decrease in tumor size and a longer $t_{1/2}$ of 5-FU. This is described as tumor trapping by Presant and Wolf (see above), where a long $t_{1/2}$ in the tumor correlates with response to treatment (decrease in tumor size). However, they found this tumor trapping in large superficial tumors and did not investigate small tumors surrounded by tissue with a large proportion of normal liver tissue. In the group with a larger contribution of the metastases to the ^{19}F-MRS signal (group b), there was a correlation between increase in tumor size and higher C_{max} of catabolites. This could mean that a higher degradation of 5-FU into catabolites correlates with a poorer response to treatment (increase in tumor size). Catabolites are thought to be produced in the normal liver and not in tumor tissue, where they appear by recirculation [17].

Although some correlations of ^{19}F-MRS parameters with change in tumor size were found, a useful and clear parameter for response to treatment was not discovered in this study. An explanation can be the volume of metastases in the field of view of the coil, which was sometimes very low (mean $V_m = 24\,cm^3$). Especially in group a (mean $V_m = 13\,cm^3$), but also in group b (mean $V_m = 78\,cm^3$), there was a large contribution of normal liver tissue to the measurement volume, also because measurements were performed not localized. This is supported by the fact that often a considerable FBAL signal was measured. The spectral parameters C_{max} and AUC, when analyzed for patients with small volumes of metastases, mainly reflect processes in the normal liver tissue. Another explanation for the lack of finding a clear correlation of our parameters with response could be the timing of the ^{19}F-MRS study. ^{19}F-MRS was performed at the sixth weekly 5-FU infusion, i.e. at the time of the radiographic evaluation. This timing was chosen in order to be able to correlate both parameters with each other. However, after 5 weeks of 5-FU treatment it is possible that we only monitor the remaining resistant cell lines in all cases.

In conclusion, the clinical application of ^{19}F-MRS of 5-FU was investigated in a random group of patients with liver metastases of colorectal cancer, treated with weekly 5-FU bolus injections with the aim to uncover aspects of the uptake and metabolism of 5-FU. Patients were not selected for the presence of very large metastases close to the surface of the liver. A correlation (C_{max} of catabolites) with response to treatment was found with ^{19}F-MRS in the group with a larger contribution of the metastases to the ^{19}F-MRS signal. However, this was not obvious in the group with a smaller contribution of the metastases, probably because of a significant contribution of normal liver tissue to the observations. In further ^{19}F-MRS studies of 5-FU the selection of patients with specific location of metastases could be considered [16] or the performance of localized ^{19}F-MRS [10,13].

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