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Noninvasive fluorine-19 NMR study of fluoropyrimidine metabolism in cell cultures of human pancreatic and colon adenocarcinoma*

Marie-C. Malet-Martino[1], Françoise Faure[2], Jean-Pierre Vialaneix[1], Claude Palevody[2], Etienne Hollande[2], and Robert Martino[1]

[1] Laboratoire des IMRCP, UA CNRS 470, Université Paul Sabatier, 118, route de Narbonne, F-31062 Toulouse Cédex, France
[2] Laboratoire de Biologie Cellulaire, Université Paul Sabatier, 38, rue des Trente-Six Ponts, F-31400 Toulouse, France

Summary. Fluorine-19 NMR spectrometry was used to monitor the metabolism of two antineoplastic fluoropyrimidines, 5-fluorouracil (5FU) and 5'-deoxy-5-fluorouridine (5'dFUrd), in cell cultures of human pancreatic (Capan-1) and colon (HT-29) adenocarcinoma. The preliminary results showed, for the two tumor cell lines treated with 5FU, the presence in nonperfused cells of three signals corresponding to intracellular metabolites: 5FU, F-nucleotides and F-nucleosides. When the cells were perfused only the signals of F-nucleotides and 5FU were present. The F-nucleosides observed during the analysis of the nonperfused cells came from the conversion of F-nucleotides. During the NMR recording of Capan-1 cells at 37 °C the first metabolite of the catabolic pathway of 5FU, 5,6-dihydro-5-fluorouracil, occurred. At the beginning of the NMR recording of Capan-1 cells treated with 5'dFUrd, two signals corresponding to F-nucleotides and F-nucleosides (consistent with 5'dFUrd) were observed; during the analysis, a supplementary signal corresponding to 5FU appeared. Even after pretreatment with methotrexate the signal of 5FU incorporated into RNA was not detected. Our experiments, performed in attempts to observe the signal of the ternary complex between thymidylate synthetase (TS), 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) and 5,10-methylene-tetrahydrofolate (5,10-CH_2FH_4), allowed detection in some cases of a broad signal, whose chemical shift was similar to that reported in the literature following incubation of TS with FdUMP and 5,10-CH_2FH_4, but our results were not always reproducible.

Introduction

Among the wide range of techniques which allow biochemical or biophysical measurements, NMR spectroscopy causes the least disturbance, as it is inherently nondestructive and noninvasive. With the elemental composition of most biological materials, there are many isotopes available for NMR biological work (e.g., ^{1}H, ^{31}P, ^{13}C, ^{23}Na). It is of great interest to the pharmacologist to apply fluorine-19 NMR (^{19}F NMR) analysis to metabolic studies of fluorinated xenobiotics, since (a) the ^{19}F nucleus has favorable NMR characteristics: ½ spin, 100% natural abundance, high sensitivity, wide chemical shift range; (b) there are no background signals from endogenous compounds, the level of fluorine being generally undetectable by ^{19}F NMR; and (c) numerous fluorinated drugs are used in therapeutics.

In previous papers, we reported a study of the metabolism of a new fluoropyrimidine, 5'-deoxy-5-fluorouridine (5'dFUrd), a prodrug of 5-fluorouracil (5FU), in human biofluids using ^{19}F NMR. This study allowed us to propose a catabolic pathway for this drug [13, 14]. One extension of this work is follow up of the anabolic fate of fluoropyrimidines in tumor cells with ^{19}F NMR.

The biochemical mechanisms of action of the most widely used fluoropyrimidine, 5FU, have been extensively studied (for a review, see [9]). The anabolic metabolism of 5FU in tumor cells is complex and leads to (a) the inhibition by 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP) of thymidylate synthetase activity and consequently of DNA synthesis, by way of depletion of the thymidine 5'-triphosphate pool; and (b) the incorporation of 5FU into RNA following the metabolic production of 5-fluorouridine-5'-triphosphate (FUTP) (Fig. 1). The mechanism of 5'dFUrd cytotoxicity is directly analogous to that reported for 5FU [1].

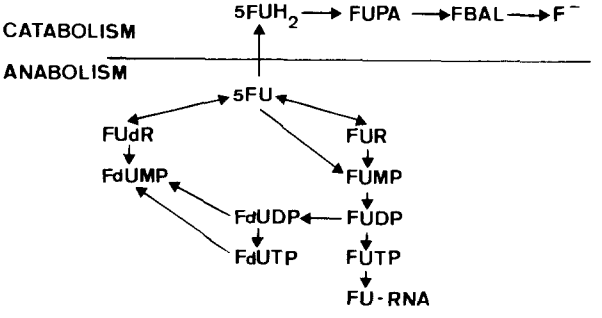

Fig. 1. Pathways of metabolism of 5-fluorouracil.
5FU, 5-fluorouracil; *FUR*, 5-fluorouridine; *FUdR*, 5-fluoro-2'-deoxyuridine; *FUMP, FUDP, FUTP*, 5-fluorouridine-5'-mono-, di-, triphosphate; *FdUMP, FdUDP, FdUTP*, 5-fluoro-2'-deoxyuridine-5'-mono-, di-, triphosphate; *5FUH*$_2$, 5,6-dihydro-5-fluorouracil; *FUPA*, α-fluoro- β-ureidopropionic acid; *FBAL*, α-fluoro-β-alanine; *F*$^-$ fluoride ion

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Offprint requests to: R. Martino

^{19}F NMR has already been used to follow the metabolism of 5FU in *Escherichia coli* cells [8] or in situ in tumors implanted in mice [19], but not in tumor cells. Studies on drug metabolism in cell cultures do not give any information about the uptake or fate of the drug in an intact tumor [19]; nevertheless, they have the advantage of direct contact of the drug with its target and allow study of the drug metabolism in a homogenous cellular population.

Since the fluorinated pyrimidines are widely used in the treatment of human solid tumors, especially of the gastrointestinal tract, we chose to monitor the metabolism of two fluoropyrimidines (5FU, 5'dFUrd) in cell cultures of human pancreatic (Capan-1) and colon (HT-29) adenocarcinomas. This paper reports our preliminary results.

Materials and methods

Drug and chemicals. 5'dFUrd, 5-fluorouridine (FUR), 5-fluoro-2'-deoxyuridine (FUdR), and 5,6-dihydro-5-fluorouracil ($5FUH_2$) were generously supplied by Hoffmann-La Roche, Basel, Switzerland. 5FU, FdUMP, and folinic acid were produced by Sigma. 5,10-Methylenetetrahydrofolate ($5,10\text{-}CH_2FH_4$) was prepared according to Moran et al. [15]. Methotrexate was a gift from Specia Laboratories, Paris, France. Sodium 4-fluorobenzoate (FBEN) was used as a reference for quantification; it was prepared by titrating 4-fluorobenzoic acid (Fluka) with NaOH solution. The relaxation reagent, chromium (III) acetylacetonate [$Cr(acac)_3$], was produced by Spectrométrie Spin Techniques.

Cell culture. Capan-1 cells, a human pancreatic adenocarcinoma cell line, were obtained from the Tissue Culture Association. This cell line was established by Dr. J. Fogh from a liver metastasis of a pancreatic adenocarcinoma grown in a 40-year old male patient [12]. Cells were grown in RPMI medium 1640 (Grand Island Biological Co.) supplemented with 15% fetal calf serum (FCS) (Grand Island Biological Co.), penicillin (200 IU/ml), streptomycin (0.25 µg/ml) and fungizone (0.25 µg/ml). Stock cultures were passaged every 10 days after a 2-min incubation with a trypsin-EDTA solution; the culture medium was replaced every other day. Cells at an initial density of 5×10^5 cells/ml were grown for 7 days in 75-cm^2 sterile flasks (Costar) to approximately 2×10^6 cells/ml.

HT-29 cells, a human colon adenocarcinoma cell line, were obtained from Dr J. Fogh at the Sloan-Kettering Institute for Cancer Research (Rye, NY). Cultures were maintained in Dulbecco medium (Grand Island Biological Co.) supplemented with 25 m*M* glucose, 10% FCS, penicillin (200 IU/ml), streptomycin (0.25 µg/ml), and fungizone (0.25 µg/ml). Stock cultures were passaged every 10 days after incubation with a trypsin-EDTA solution; the culture medium was replaced every day. Cells at an initial cell density of 10^5 cells/ml were grown for 12 days in 75-cm^2 sterile flasks (Costar) to approximately 2×10^6 cells/ml.

Capan-1 and HT-29 cells were tested for Mycoplasma contamination monthly and consistently found to be negative during the course of these experiments.

Preparation of samples for ^{19}F NMR analysis. Cells grown to a density of approximately 2×10^6 cells/ml were incubated with 5FU at two doses (0.385 m*M* and 3.85 m*M*) for 4 h or with 5'dFUrd (6.1 m*M*) for 2 h at 37 °C. After repeated pipetting to dissociate cell aggregates (Capan-1) or after trypsinization (HT-29), cells were harvested by centrifugation (700 *g* for 1 min) at ambient temperature. They were then washed twice with drug-free medium to remove any extracellular drug, resuspended in the minimum of 5% glucose isotonic solution for nonperfused studies or culture medium supplemented with 10% (HT-29) or 15% (Capan-1) FCS for perfused recordings, and then immediately placed into an NMR tube.

Capan-1 cells were also exposed to methotrexate (1.3 m*M*) for 3 h. The drug-containing medium was then removed and replaced by medium containing 5FU (3.85 m*M*). Cells were incubated with 5FU for 2 h. They were then treated as described above.

Capan-1 cells were also incubated with folinic acid (0.2 m*M*) together with 5FU (3.85 m*M*) for 4 h. They were then treated as described above.

In some experiments, prior to the NMR analysis, the Capan-1 cells were disrupted by sonication (MSE sonicator) for 30 s. To prepare cytosol, the suspension was then centrifuged at 105000 *g* for 30 min at 4 °C. In some cases $5,10\text{-}CH_2FH_4$ alone (4 m*M*) or with FdUMP (1 m*M*) was added either to the crude sonicate or to the cytosol.

In some experiments, an internal perfusion system was used to maintain cell viability; in these cases, the cell pellet was perfused with culture medium supplemented with FCS at a rate of 0.03 ml/min. The cell viability was determined using the trypan blue (0.5%) exclusion test.

Analytical method. ^{19}F NMR spectra were recorded at 250 MHz on a CAMECA 250 FT spectrometer interfaced to a 16K memory NICOLET 80 computer. Spectra were run without proton decoupling and with no frequency field lock in 5-mm-diameter NMR tubes. The resonance positions were measured from the H_2O proton signal, which is always positioned at the same frequency for any sample, and referenced to an external standard CF_3COOH (0.5 g/100 ml aqueous solution, 25 °C) resonance peak. The instrumental settings were established as follows: probe temperature: 4 °C or 37 °C; sweep width: 33333 Hz; pulse width: 1.6 µs; recycling time: 2 s; number of scans: 6000–30000; computer resolution: 4.1 Hz/point; line broadening caused by exponential multiplication: usually 20 Hz (4 Hz in some experiments); receiving filter: out. The magnetic field was shimmed by using the ^1H NMR resonance of water observed in the continuous wave mode.

In the nonperfused studies quantification was performed. The spectrometer has been modified to obtain sufficient spectral density of the radiofrequency pulse and a correct receiver bandwith over such a large chemical shift range as 130 ppm [4]. For the purpose of quantitation, capillary containing the FBEN reference [aqueous solution of FBEN (74 m*M*) and $Cr(acac)_3$ (2.7 m*M*)] was put into the NMR tube. The spin-lattice relaxation time (T_1) of FBEN in the aqueous solution is 1.4 s. Using a pulse width of 1.6 µs, we verified that a recycling time of 2 s, allowed good quantification of this compound. We did not measure the T_1 of the different intracellular fluorinated metabolites, but we verified that in an experiment at 4 °C, where there is no evolution of the 5FU signal, a recycling time >2 s did not modify the intensity of this 5FU signal.

The different fluorinated metabolite concentrations were determined from the intensities of their respective

NMR signals. These intensities were estimated by comparing the expanded areas of the NMR signals (expanded scale: 20 Hz/cm) with that of FBEN. The areas were determined after cutting and weighing the different signals. We verified that the total concentration of the intracellular fluorinated metabolites did not change during the NMR recording. We could therefore estimate the variation of their respective concentrations as a function of the NMR recording time.

The accuracy of the NMR assay is 5%–10%, depending upon the signal-to-noise ratio of the peaks.

Results

Intracellular unbound fluorinated metabolites

Nonperfused tumor cells. In Fig. 2a, we present the spectrum obtained when Capan-1 cells were treated with 5FU (3.85 m*M*) for 4 h and recorded at 4 °C over 3 h without perfusion. Two major peaks are observed, one located at $\delta = -93.9$ ppm, consistent with intracellular unmetabolized 5FU, the other at $\delta = -89.6$ ppm, attributed to free intracellular F-nucleotides. There is also a small shoulder at $\delta = -90.6$ ppm, corresponding to F-nucleosides (FUR or FUdR). Peak assignments were obtained from an experiment in which the cells were disrupted by sonication and the homogenate obtained spiked with small amounts of standard compounds (5FU, FUR, FUdR and FdUMP). The ^{19}F chemical shifts (δ) of FUR and FUdR were identical, and those of the various F-nucleotides very similar [19]. It was therefore only possible to assign the resonances at $\delta = -90.6$ ppm and $\delta = -89.6$ ppm to F-nucleosides and F-nucleotides, respectively. During the NMR recording, the F-nucleotide peak decreased in favor of the F-nucleoside signal, the 5FU signal being constant (Figs. 2b and 3a).

We also incubated Capan-1 cells with a 5FU dose only one-tenth as high (0.385 m*M*); 15 h of NMR recording was necessary to attain a spectrum with a correct signal-to-noise ratio. In these circumstances the spectrum at 4 °C was quite similar to that shown in Fig. 2b, except that the intensity of the unmetabolized 5FU signal was much lower.

The ^{19}F NMR spectra of the human colon adenocarcinoma cells (HT-29) treated with 5FU (3.85 m*M*) for 4 h and recorded at 4 °C were quite comparable to those already described for Capan-1 cells (Fig. 2c). In the first recording (0–3 h), F-nucleotides and unmetabolized 5FU were observed; during the NMR analysis the F-nucleosides signal became visible.

When Capan-1 cells were incubated with 5'dFUrd (6.1 m*M*) for 2 h, the spectrum obtained after 6 h of NMR recording at 4 °C showed the F-nucleotide peak ($\delta = -89.7$ ppm) and a second signal at $\delta = -90.7$ ppm, which probably contained contributions from unmetabolized 5'dFUrd and F-nucleosides giving resonances at the same chemical shift. The fact that this signal decreased with time suggests that it contained primarily 5'dFUrd, which was partially converted to 5FU during the NMR analysis.

In all the spectra obtained at 37 °C, the chemical shifts of the different intracellular fluorinated metabolites were deshielded by 0.3–0.5 ppm with regard to the δ observed for the spectra recorded at 4 °C (this value must be ≈ 0.2 ppm up, owing to the deshielding of the H_2O proton signal when the probe temperature was raised from 4 °C to 37 °C). When Capan-1 cells treated with 5FU (3.85 m*M*) for 4 h were recorded at 37 °C, the spectrum showed a larger amount of intracellular 5FU than when they were recorded at 4 °C (Fig. 4a). During the first 6 h of NMR recording, 5FU represented $\approx 65\%$ of the fluorinated metabolites (Fig. 3b), while it represented only $\approx 30\%$ when the recording was done at 4 °C (Fig. 3a). 5FU arises from a rapid breakdown of F-nucleotides, which are totally lacking after 9 h. The proportion of F-nucleosides increased with time only very slightly but, after the first 3 h of NMR analysis, it had already reached a percentage similar to that observed between 15 and 18 h when the recording was carried out at 4 °C (Fig. 3b). During the NMR analysis at 37 °C, 5FU was converted into $5FUH_2$, the first product of the 5FU catabolic pathway, which gave a signal at $\delta = -125.7$ ppm (Fig. 4b).

For HT-29 cells treated in similar conditions a much slower evolution was noticed (Fig. 3c). On the other hand, $5FUH_2$ occurrence was not observed.

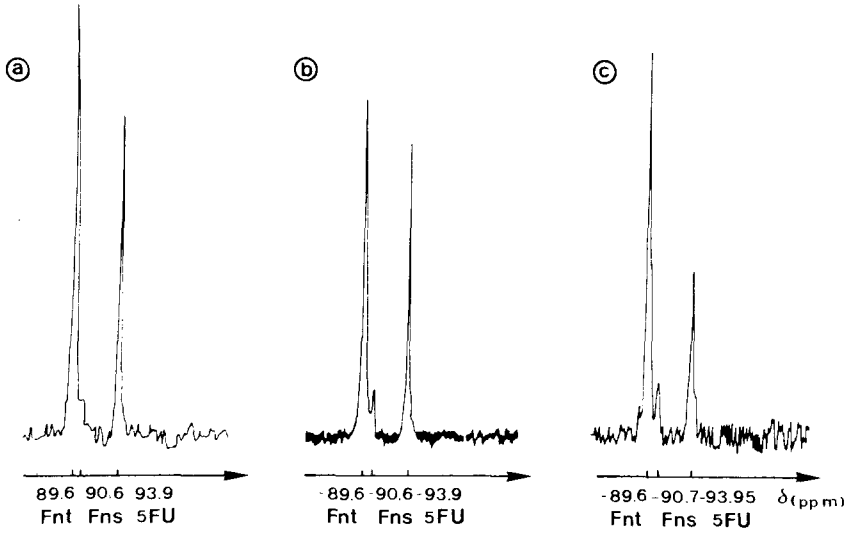

Fig. 2a–c. ^{19}F NMR spectra of Capan-1 cells **(a, b)** and HT-29 cells **(c)** treated with 5FU (3.85 m*M*) for 4 h and recorded at 4 °C without perfusion. Time of NMR recording: **a** 0–3 h, **b, c** 15–18 h. *Fnt*, F-nucleotides; *Fns*, F-nucleosides

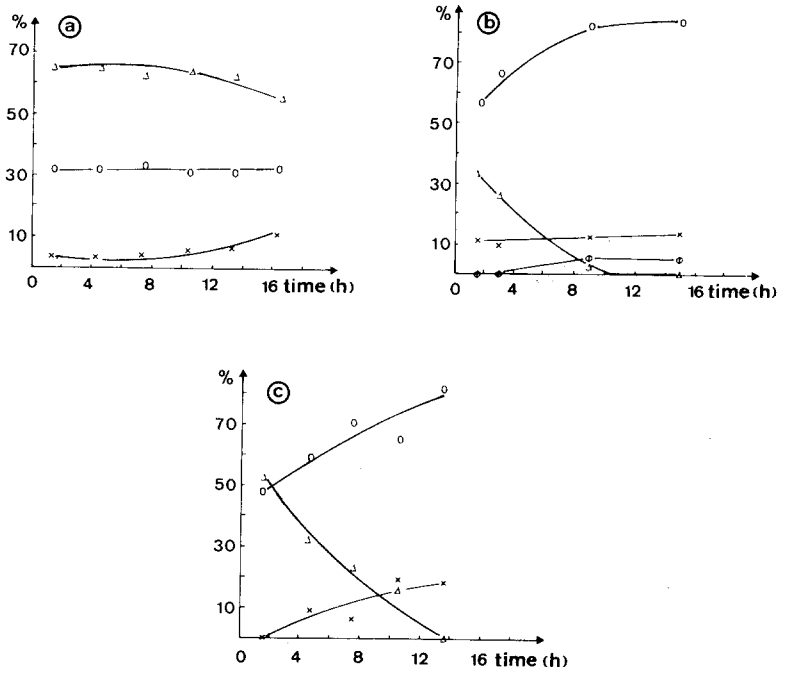

Fig. 3a–c. Variations in percentages of the different intracellular fluorinated metabolites during the ^{19}F NMR recording. **a, b** Capan-1 cells treated with 5FU (3.85 m*M*) for 4 h and recorded at 4 °C **(a)** or 37 °C **(b)**; **c** HT-29 cells treated with 5FU (3.85 m*M*) for 4 h and recorded at 37 °C. The time of each NMR recording block being 3 h, the percentages of the various metabolites are considered to be representative of the state of the metabolism 1.5 h after the beginning of each NMR recording block. Δ, F-nucleotides; O, 5FU; X, F-nucleosides; Φ, $5FUH_2$

Perfused tumor cells. We perfused the tumor cells with culture medium at 37 °C for 6 h. Viability was assessed by trypan blue exclusion and was 90% for nontreated Capan-1 cells and 50% when the cells were incubated with 5FU (3.85 m*M*, 4 h). The viability of HT-29 cells treated with 5FU (3.85 m*M*, 4 h) and perfused in the same conditions was 80%.

The spectra of Capan-1 and HT-29 cells show two signals, one attributed to 5FU ($\delta = -93.6_5$ ppm), the other to F-nucleotides ($\delta = -89.1$ ppm) (Fig. 5). During the 6-h NMR recording, there were no changes in the ratios of the two metabolites and no occurrence of the F-nucleosides signal.

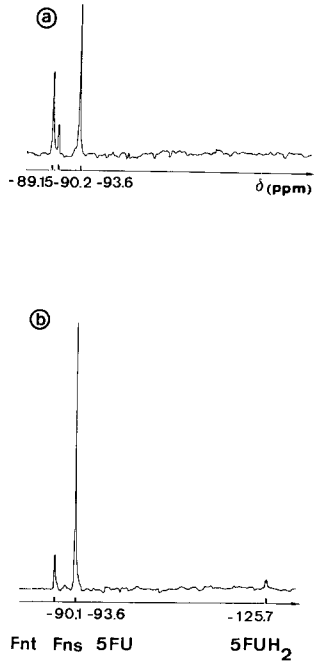

Fig. 4a, b. ^{19}F NMR spectra of Capan-1 cells treated with 5FU (3.85 m*M*) for 4 h and recorded at 37 °C without perfusion. Time of NMR recording: **a** 0–3 h, **b** 12–15 h. *Fnt*, F-nucleotides, *Fns*, F-nucleosides

Attempts to observe the 5FU fluorinated metabolites bound to macromolecules

In prokaryotic cells, the RNA-incorporated FUTP gives a very broad ^{19}F NMR signal located between 1 and 10 ppm downfield of 5FU [8, 10]; this resonance region also corresponds to that of F-nucleotides and F-nucleosides. In our experimental conditions, we were unable to detect this signal even after pretreatment with methotrexate, which is known to increase 5FU incorporation into RNA [3, 6].

FdUMP is a suicide inhibitor which forms a ternary covalent complex with the enzyme thymidylate synthetase and with the cofactor 5,10-CH_2FH_4 [9]; the native complex, when prepared in vitro, gives a ^{19}F NMR resonance at $\delta = -102.1$ ppm from external CF_3COOH [5]. When Capan-1 cells were treated with 5'dFUrd (6.1 m*M*, 2 h) and recorded at 4 °C, a large signal at $\delta = 101.0$ ppm was observed. We had thought it might correspond to the signal of the ternary complex; however, another experiment carried out in similar conditions did not allow detection of this resonance.

Due to the heterogeneity of the medium in intact cells, we supposed that this signal might have been enlarged and hence difficult to detect; we therefore studied Capan-1 cells treated with 5FU (3.85 m*M*, 4 h) and disrupted by sonication; the sonication did not induce the expected narrowing of the signals, but a broad resonance centered at $\delta = -104.4$ ppm was observed; this resonance was no longer detected after 3 h of NMR recording at 4 °C. Two subsequent experiments in the same conditions did not allow detection of this signal again.

It has been reported that maximum ternary complex formation occurs only in the presence of exogenous 5,10-CH_2FH_4 [11], indicating that endogenous cofactor le-

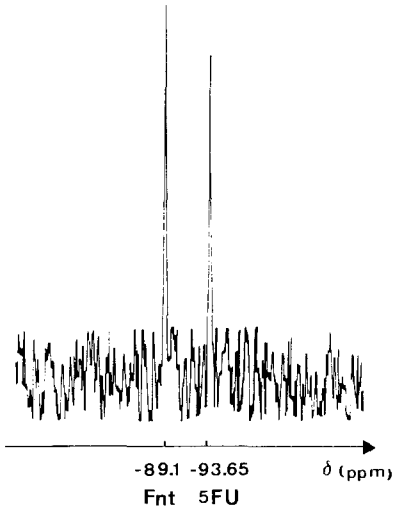

Fig. 5. ^{19}F NMR spectrum of Capan-1 cells treated with 5FU (3.85 m*M*) for 4 h and recorded at 37 °C with perfusion. Time of NMR recording: 0–2 h. *Fnt*, F-nucleotides

vels are insufficient for maximal thymidylate synthetase inhibition. We therefore added 5,10-CH_2FH_4 (4 m*M*) to 5FU-treated (3.85 m*M*, 4 h) and sonicated Capan-1 cells: in one experiment no large signal was observed; in another, a broad signal at $\delta = -102.4$ ppm was detected for 6 h at 4 °C. Two other experiments have proved to be negative: when a cell lysate or a cytosol of untreated Capan-1 cells was spiked with FdUMP (1 m*M*) and 5,10-CH_2FH_4 (4 m*M*), we were unable to detect a signal at $\delta \simeq -102$ ppm.

Potentiation of the cytotoxicity of 5FU mediated by stabilization of the enzyme-FdUMP complex has been reported following the addition of folinic acid to tumor cells in culture [7, 20]. We therefore exposed Capan-1 cells to 5FU (3.85 m*M*) in combination with folinic acid (0.2 m*M*) for 4 h; the ^{19}F NMR recording of the intact cells at 4 °C showed a signal at $\delta = -101.95$ ppm.

Discussion

Recently, numerous HPLC systems have been developed for studies of the intracellular metabolism of fluoropyrimidines [2, 16]. They permit rapid identification and quantification of the major metabolites of 5FU, but they involve the preparation of cell extracts and usually the necessity for labeled drugs. Since several intracellular metabolites of 5FU are unstable, these methods are difficult to implement and do not exactly reflect the intracellular metabolism of 5FU. We therefore thought to use the ^{19}F NMR method (which allows the study of living cells with no prior treatment and requires no labeled drugs) to monitor the metabolism of two fluoropyrimidines (5FU, 5'dFUrd) in cell cultures of human pancreatic and colon adenocarcinoma. One drawback of the ^{19}F NMR method is its low intrinsic sensitivity. We therefore had to use high concentrations of drugs to obtain sufficiently intense signals in a reasonable period of time with the CAMECA 250 spectrometer, which only takes 5-mm-diameter NMR tubes.

The ^{19}F NMR spectra of Capan-1 or HT-29 cells treated with 5FU or 5'dFUrd and recorded without perfusion generally show three signals corresponding to free intracellular fluorinated metabolites: 5FU, F-nucleotides, and F-nucleosides, The intracellular F-nucleosides, FUR and FUdR, as well as the drug 5'dFUrd, give nearly identical resonances. The signal of the F-nucleotides probably contains contributions from the different mono-, di-, and triribo- and deoxyribonucleotides [19]. This difficulty in resolving the F-nucleotides and F-nucleosides signals is one of the drawbacks of the ^{19}F NMR methodology applied to this kind of study.

Examination of the behavior of the fluorinated metabolites in tumor cells treated with 5FU and recorded without perfusion indicated a progressive conversion of the F-nucleotides signal in favor of F-nucleosides and/or 5FU; this transformation becomes more rapid with rising temperature of the NMR analysis. On the other hand, during the recording of perfused cells we found no evidence (within the limits of sensitivity of the ^{19}F NMR method) of F-nucleosides. Therefore, the F-nucleoside signal, observed as early as after the first 3 h of NMR recording of nonperfused cells, must come from the F-nucleotides breakdown. This rapid transformation stresses the necessity of rapid operation in correct conditions of cell perfusion.

In Capan-1 cells treated with 5FU (3.85 m*M*) for 4 h and recorded at 37 °C without perfusion, we noted the occurrence of a small amount ($\simeq 5\%$ of the total fluorinated metabolites) of the first product of the 5FU catabolic pathway, $5FUH_2$. We did not detect the catabolites coming from the 5FU ring opening, FUPA and FBAL. Although it is known that the catabolism of 5FU occurs in tumors but is reduced [9], ^{19}F NMR allowed observation of this only in Capan-1 cells (not in HT-29 cells) and at 37 °C in experimental conditions leading to cell death.

Concerning the 5FU active anabolites bound to macromolecules, our attempts to detect the signal of FUTP incorporated into RNA proved negative. For the ternary complex between FdUMP- 5,10-CH_2FH_4-thymidylate synthetase, our results were irregular. In some experiments, we observed a large signal at $\delta \simeq -102$ ppm. Some arguments may suggest that it corresponded to the ternary complex: (a) the similarity of the chemical shift to that reported by Byrd et al. [5]; and (b) the fact that this signal is no longer detected with time. Indeed, the half-time for dissociation of the ternary complex in a suspension of rat hepatoma cells after sonication was found to be approximately 2 h at 37 °C [21]. As in all cell lysates studied, the F-nucleotides (including FdUMP) were rapidly converted to F-nucleosides even at 4 °C, the reformation of the ternary complex was not possible. This explains why the ternary complex concentration decreased and the resonance of this entity was no longer detected after a short period of NMR recording.

However, even in conditions which should theoretically have increased the formation of the ternary complex (increase of folate cofactor level), its signal was not always observed. The aleatory feature of our results can be explained by the fact that the ternary complex concentration was at the limit of the concentration detectable with ^{19}F NMR in our experimental conditions, and/or by uncontrolled modifications of the physiological state of Capan-1 cells. Nevertheless, the occasional detection of this signal implies a high concentration of thymidylate synthetase in Capan-1 cells, much higher than that reported in the litera-

ture for other tumor cells [11, 18]. An overproduction of thymidylate synthetase in 5-fluorodeoxyuridine-resistant mouse fibroblasts has recently been described [17], and Kyriazis et al. have shown the resistance of Capan-1 tumor to 5FU [12]. Our results suggest that this drug resistance is not associated with the failure of Capan-1 cells to convert 5FU to F-nucleotides, but may be due to high levels of thymidylate synthetase activity [11].

In conclusion, despite significant drawbacks (low sensitivity, nonresolution of the signals of the various F-nucleotides or F-nucleosides), the noninvasive ^{19}F NMR method can provide useful information on the metabolism of the fluoropyrimidines in tumor cells. With a better performance spectrometer, it will be possible to monitor the incorporation, the fate and the mechanisms of action of fluorinated antineoplastic drugs directly in the NMR probe.

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