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5-Fluorouracil metabolism and cytotoxicity after pre-treatment with methotrexate or thymidine in human hypopharynx and colon carcinoma xenografts: a ^{19}F-nuclear magnetic resonance spectroscopy study in vivo

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Abstract The metabolism of 5-fluorouracil (5-FU) was monitored non-invasively in two xenografts, a hypopharynx carcinoma and a colon carcinoma (CSM) by ^{19}F-magnetic resonance spectroscopy following an i.v. bolus injection of 130 mg kg^{-1} 5-FU. Both the level of fluoronucleotides (FNuc) and the tumour growth delay were significantly higher in the CSM colon carcinoma than in the hypopharynx carcinoma (both parameters, $P < 0.001$). Administration of 100 mg kg^{-1} methotrexate (MTX) at 15 h before treatment with 5-FU caused a significantly increased conversion of 5-FU to FNuc in both tumours ($P < 0.05$) as compared with the application of 5-FU alone. However, only in the CSM tumour was a significantly increased growth delay ($P < 0.01$) observed. Pre-treatment of both xenografts with 400 mg kg^{-1} thymidine enhanced the conversion of 5-FU to FNuc in both tumours. In the CSM tumour this treatment modality caused a significantly ($P < 0.05$) higher growth delay as compared with the results obtained with 5-FU alone, whereas in the hypopharynx carcinoma the additional application of thymidine caused no significant change in tumour growth. It is known that both thymidine and MTX can reduce the DNA-directed cytotoxicity of 5-FU, whereas the RNA-directed cytotoxicity is increased. It is concluded that the DNA-mediated toxicity may be more important in the hypopharynx carcinoma than in the CSM colon carcinoma. As a consequence, pre-treatment with MTX or thymidine enhances FNuc formation, although only in the CSM carcinoma is there an increased tumour growth delay. Thus, in the hypopharynx carcinoma the measurement of FNuc did not serve as a predictor for the treatment efficacy of the combined treatment modality. Pre-treatment with MTX did not influence the catabolism of 5-FU, whereas thymidine actually prolonged the half-life of 5-FU without α-fluoro-β-alanine becoming detectable.

Key words ^{19}F-NMR spectroscopy · 5-FU · Combined treatment modality

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Introduction

5-Fluorouracil (5-FU) exerts its cytotoxicity only after being metabolised to the 5-fluoro-(2′-deoxy)uridine-5′ nucleotides (FNuc) 5-fluorouridine-5′-triphosphate (FUTP) and 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP). The liver is the main site of catabolism of 5-FU, via 5-fluorodihydrouracil (FDHU) and α-fluoro-β-ureidopropionic acid (FUPA), to α-fluoro-β-alanine (FBAL) [13]. FUTP is incorporated into RNA, leading to interference with the maturation of nuclear RNA, whereas FdUMP forms a complex with thymidylate synthase (TS) and 5,10-methylenetetrahydrofolic acid (THF), inhibiting DNA synthesis (Fig. 1) [13]. The relative importance of each cytotoxic mechanism may be dependent upon varying patterns of intracellular 5-FU metabolism. The conversion of 5-FU by tumour cells into other fluorine compounds can be monitored in vivo in a non-invasive manner by ^{19}F-nuclear magnetic resonance (NMR) spectroscopy. Thus, this method may allow a prognosis concerning the expected cytotoxic effect of therapy with 5-FU, and several investigators have predicted the cytotoxicity of 5-FU by measuring the integral of a peak with a pH-dependent chemical shift of 4.5–5 ppm downfield of 5-FU [14–16, 20]. This peak, which is called FNuc in the present paper, contains contributions of the cytotoxic anabolites FUTP and FdUMP. However, due to their structural similarity, all fluorine-containing nucleotides (e.g. FUTP, FUDP, FUMP, FdUMP) contribute to the FNuc peak. Furthermore, the FNuc peak contains contributions from other fluorouridi-

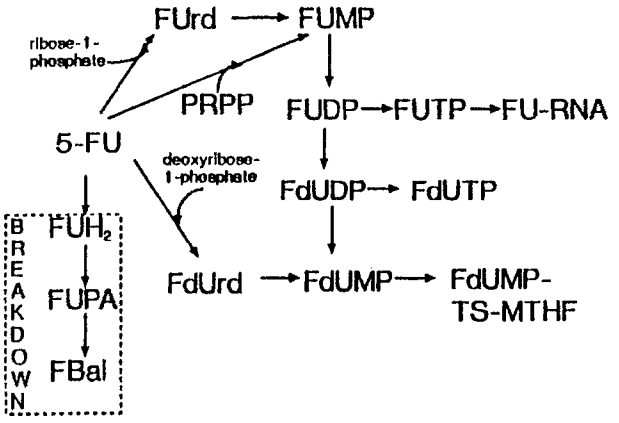

Fig. 1 Enzymatic pathway of 5-FU metabolism, including 5-fluorouracil (*5-FU*); 5-fluorouridine (*FUrd*) and its 5′-monophosphate (*FUMP*), -diphosphate (*FUDP*), and -triphosphate (*FUTP*); 5-fluoro-2′-deoxyuridine (*FdUrd*) and its monophosphate (*FdUMP*), diphosphate (*FdUDP*), and triphosphate (*FdUTP*): 5-FU incorporated into RNA (*FU-RNA*); complex of FdUMP with thymidylate synthase (*TS*) and 5,10-methylene-tetrahydrofolic acid (*MTHF*); (*FdUMP-TS-MTHF*); 5,6-dihydrofluorouracil (FUH_2); α-fluorouredo-β-propionic acid (*FUPA*); and α-fluoro-β-alanin (*FBal*)

lates as well as 5-fluorouridine diphosphate glucose [10, 14]. Finally, FdUMP is present in very low concentrations and may be bound to thymidylate synthetase [7], and, thus, be undetectable by NMR. Accordingly, the FNuc peak may be a fairly unprecise measure of the cytotoxicity of 5-FU, especially in tumours or cell lines in which the DNA-directed cytotoxicity of FdUMP is important.

To enhance the activation of 5-FU, various agents modulating the drug's metabolism have been evaluated. One of these agents is methotrexate (MTX), which causes inhibition of de novo purine synthesis through the depletion of intracellular reduced folates, leading to a rise in the concentration of cytoplasmatic 5-phosphoribosyl-1-pyrophosphate (PRPP) [5]. In tumour cells, increased PRPP concentration results in a greater conversion of 5-FU to FUMP and FUTP [5]. Another agent that enhances the incorporation of 5-FU into RNA is thymidine, which acts by suppressing the catabolism of 5-FU [12, 25]. Both drugs enhance the RNA-directed cytotoxicity of 5-FU, whereas the DNA-directed toxicity is diminished. ^{19}F-NMR spectroscopy has previously been shown to be capable of predicting the cytotoxicity of 5-FU used as a single agent or in combination with MTX or thymidine [14–16, 19, 20].

In the present study, the modulation of 5-FU metabolism using the aforementioned drugs was investigated by ^{19}F-NMR spectroscopy in two xenografted tumour lines grown on athymic nude mice. In a xenografted human colon carcinoma, MTX and thymidine enhance the anti-tumour activity of 5-FU. In contrast, in a xenografted hypopharynx carcinoma, co-administration of thymidine reduces the growth delay caused by 5-FU, whereas MTX enhances 5-FU cytotoxicity in a less than additive manner as compared with the results of the single-agent treatment. The aim of this study was to determine whether ^{19}F-NMR spectroscopy is capable of predicting the treatment efficacy in the two tumour lines when 5-FU cytotoxicity is modulated by the aforementioned drugs.

Materials and methods

Drugs and chemicals

5-FU and thymidine were obtained from Sigma Chemical Co. (USA) and methotrexate (MTX) was supplied by Lederle Arzneimittel (Germany).

Tumours and chemotherapy

The investigations were performed on a moderately differentiated carcinoma of the hypopharynx and a moderately differentiated colon carcinoma (CSM), both having been removed surgically from untreated patients. The tumours were heterotransplanted serially in male athymic mice as described elsewhere [22]. Tumour volumes were estimated by caliper measurement according to the formula for the volume of ellipsoids (0.5 × length × width^2). At the time of the experiments of the present study the doubling time of tumour volume amounted to about 8 days in the hypopharynx and 5 days in the CSM xenografts. The volume of the tumours was approximately 1.2 cm^3 when they entered the study at approximately 3 weeks after heterotransplantation. This tumour size was necessary to obtain sufficiently good ^{19}F-NMR spectra. After treatment of the tumours, growth delay or even shrinkage of the tumours occurred. The effect of the agents on tumour growth was determined by measuring the time the tumours took to grow to twice the starting volume. This time was compared with that required for untreated tumours and was expressed as the tumour growth delay.

MTX (100 mg kg^{-1}) was applied i.p. at 12 h and thymidine (400 mg kg^{-1} i.p.) at 1 h before 5-FU (130 mg kg^{-1} i.v.) administration. The rationale for these particular doses was to achieve a good pharmacological effect of the treatment schedules and tolerable toxicity for the animals. Comparable doses in ^{19}F-NMR studies have been used before [14, 16, 19].

^{19}F-NMR spectroscopy

All spectra were obtained at 338.8 MHz using an 8.5 T 8-cm vertical bore magnet (Bruker AM 360). An 11-mm, one-turn surface coil was used. ^{19}F-NMR spectra were obtained by placing the coil on the tumour in contact with the skin. The magnet was shimmed on water-proton signals. Water-line widths ranged between 60 and 115 Hz. Spectral acquisition parameters included a spectral width of 20 kHz, a pulse interval of 1 s, and 600 acquisitions. Each free induction decay was processed by 40-Hz line broadening. In one experiment a tumour with a volume of approximately 1.0 cm^3 was ligated from the blood circulation so as to ascertain that the underlying tissue did not contribute to the tumour-derived NMR spectral data. The 5-FU signal obtained after the injection of drug into the tail vein of the mouse amounted to less than 10% of the signal obtained without tumour ligation. In experiments using 5-FU as a single agent, small reference samples of fluorobenzene were axially positioned at 8 mm from the center of the surface coil. However, in experiments in which 5-FU was applied in combination with thymidine or MTX, no reference sample was used. In this case the FNuc/5-FU ratio was used as a measure of the influence of the combined therapy modality on 5-FU metabolism. This ratio gives an estimate of the efficiency of the conversion of 5-FU to FNuc. The mice were anaesthetised by low doses of a mixture of Valium and Hypnorm (7 mg kg^{-1} fluanison, 0.14 mg kg^{-1} fentanyl base and 7 mg kg^{-1} diazepam before the tail vein was punctured. This mode of anaesthesia was used since some mice died in preliminary experiments when pentobarbital or Ketanest/Rompun was applied. Simultaneously with the administration of 5-FU (130 mg kg^{-1} i.v.), given as a bolus injection over a period not exceeding 1 min, acquisition of the

spectra was started. The spectra were collected at consecutive 10-min intervals (600 scans), each experiment lasting 70 min.

Diazepam is known to inhibit the influx of uridine and its derivatives into cells [18]. For this reason, control studies were performed to determine whether the tumours investigated in this study exhibited a different 5-FU uptake in mice anaesthetised with diazepam/Hypnorm in comparison with non-anaesthetised mice. In ten animals (five for each xenograft), no significant difference in 5-FU uptake was found.

Each free induction decay was processed by 40-Hz line broadening. The peaks in the ^{19}F- NMR spectra were fitted to Lorentzian curves. In those cases where fluorobenzene was used as a reference, the ratios of the tumour-derived fluorine signals to the reference signal were calculated from the areas under the curves. To calculate the efficiency of the formation of FNuc from 5-FU, the FNuc/5-FU$_0$ ratio was used, where FNuc is the plateau level of FNuc measured after 50–60 min and 5-FU$_0$ is the initial concentration of 5-FU. The spectra shown in Fig. 2 and evaluated in Figs. 3 and 4 are the sums of two consecutive 10-min spectra for the labeled period. This technique is equivalent to a two-point smoothing procedure for the 10-min data, but it was necessary to achieve a reasonable signal-to-noise ratio for FNuc in the experiments. The half-time shown for 5-FU in Table 3 was calculated from the original spectra ($n = 7$ time points).

Differences in group means were analyzed by Student's two-tailed t-test (independent samples). Changes in tumour volume were analyzed by the t-test difference method (two-tailed, dependent samples).

Results

Table 1 presents the growth delay observed after treatment of the two carcinoma xenografts with 5-FU and some other compounds. Application of 5-FU alone induced a significant growth delay in the CSM colon carcinoma ($P < 0.001$) and the hypopharynx carcinoma ($P < 0.01$) as compared with control tumours. The growth retardation seen in the CSM colon carcinoma was significantly higher ($P < 0.001$) than that observed in the hypopharynx carcinoma. The additional application of 100 mg kg^{-1} MTX at 12 h or 400 mg kg^{-1} thymidine at 1 h before 5-FU administration produced a significantly longer growth delay in the CSM carcinoma xenografts than did treatment with 5-FU alone (Table 1). In contrast, in the hypopharynx carcinoma, co-administration of MTX enhanced the growth suppression in a non-significant manner and thymidine even had a slight, albeit non-significant, antagonistic effect on the tumour cytotoxicity induced by 5-FU (Table 1). On the other hand, however, MTX and thymidine considerably increased the general toxicity of 5-FU (Table 1). In both xenografts, administration of MTX alone caused a higher growth retardation than did administration of thymidine alone (Table 1).

Serial ^{19}F-NMR spectra recorded for the CSM tumour after i.v. bolus injection of 130 mg kg^{-1} 5-FU are shown in Fig. 2. The resonances detectable were 5-FU (A; 0 ppm), FNuc (B; 4.8 ppm), FUPA (C; –17.3 ppm) and FBal (D; –19 ppm). The catabolite FUH_2 or fluoronucleosides were never detected in the carcinoma xenografts. Figures 3a and 4a give a summary of the results obtained in 14 tumour-bearing animals that received this dose of 5-FU. In the subsequent acquisitions, there was a concomitant decrease in 5-FU and an increase in FNuc concentrations. The maximal FNuc level was usually reached at 50–60 min after 5-FU application. The pattern of drug metabolism that was observed when 5-FU was applied to the hypopharynx carcinoma was quite similar to that observed in the CSM xenograft (Figs. 3b, 4b). However, the amount of 5-FU detectable in the tumours and measured as the initial 5-FU/reference (Ref) ratio was significantly higher in the CSM xenograft (0.165 ± 0.044) than in the hypopharynx carcinoma (0.124 ± 0.039, $P < 0.05$). In addition, the FNuc/5-FU$_0$ ratio, which gives a measure of the efficiency of the formation of FNuc from 5-FU, was significantly higher in the CSM colon carcinoma than in the hypopharynx carcinoma xenografts (0.24 ± 0.11 versus 0.13 ± 0.0068, $P < 0.01$; Table 2). This ratio is determined by the initial 5-FU concentration and the plateau level of FNuc measured at 50–60 min after substance application. As a consequence of the better uptake of 5-FU and the higher fraction of 5-FU being anabolised to FNuc in the CSM colon carcinoma in comparison with the hypopharynx carcinoma, the level of FNuc was clearly lower in the latter tumour (0.045 ± 0.016 versus 0.017 ± 0.004 FNuc/Ref ratio, $P < 0.001$; calculated from Fig. 3). Co-administration of MTX or thymidine resulted in an increase in the FNuc/5-FU$_0$ ratio in both tumours, this increase not being significant in the case of thymidine in the hypopharynx carcinoma xenograft (Table 2).

Concerning the catabolites FUPA and FBal, the intratumoural levels of both metabolites showed considerable variation between individual tumours in both carcinoma types investigated (Fig. 4). The resonance of FUPA usually appeared after 30 min and reached its maximum after 40–60 min, whereas after pre-treatment with thymidine, the level of FUPA was still rising after 70 min (data not

Table 1 Tumour growth delay, number of animals and weight change recorded after treatment of nude mice bearing the hypopharynx carcinoma (Hyp) and the CSM colon carcinoma with either 5-FU (130 mg/kg), 5-FU + methotrexate (100 mg/kg), 5-FU + thymidine (400 mg/kg), thymidine alone, or methotrexate alone. The standard deviation is given in parentheses. The effect of the agents on tumour growth was determined by measuring the time the tumours took to grow to twice the starting volume. This time was compared with that required for untreated tumours and was expressed as the tumour growth delay. The weight change was determined on day 0 and day 7 after treatment and is the sum of the values recorded for the two treatment groups

	Growth delay, days (Hyp)	n (Hyp)	Growth delay, days (CSM)	n (CSM)	% Change in weight
5-FU	3.8 (2.1)	12	6.3 (1.9)	14	–7.8 (2.2)
5-FU/ methotrexate	5.2 (2.1)	6	9.7 (2.4)**	5	–13.5 (2.9)
5-FU/ thymidine	2.9 (1.6)	5	8.7 (2.5)*	6	–15.8 (2.7)
Methotrexate	2.0 (1.7)	6	1.8 (1.7)***	6	–2.6 (1.2)
Thymidine	0.8 (1.5)**	5	0.9 (1.9)***	7	–1.5 (1.4)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared with treatment with 5-FU alone

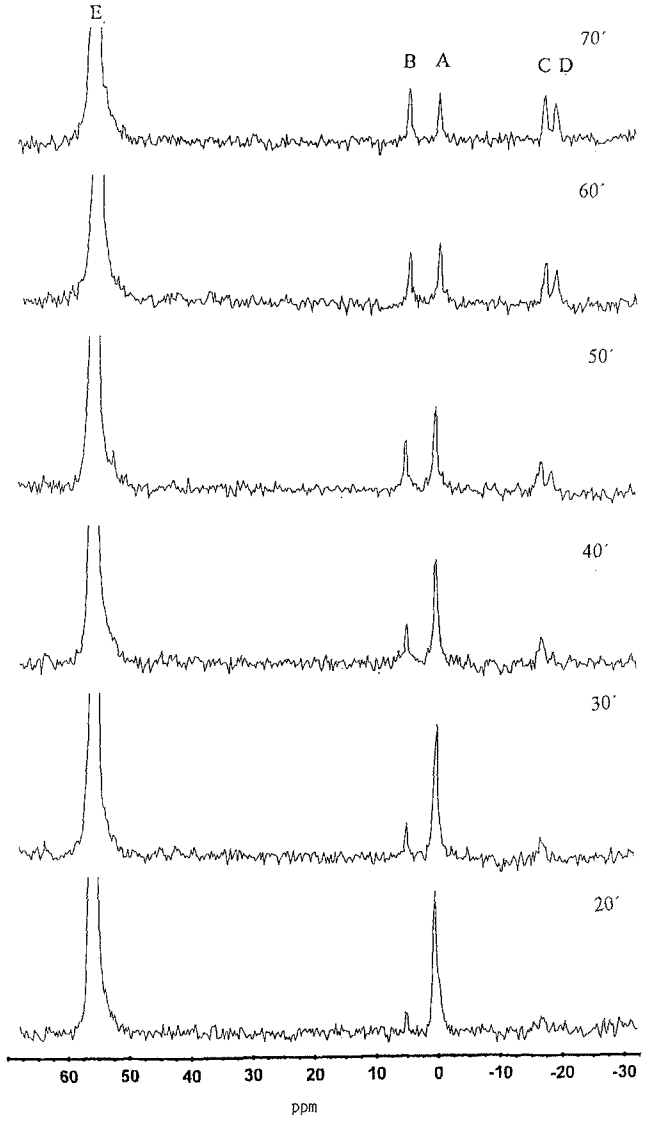

Fig. 2 Sequential ^{19}F NMR spectra recorded for the CSM colon carcinoma xenograft during the first 70 min following an i.v. bolus injection of 130 mg kg^{-1} 5-FU at time zero. For each 20-min acquisition, the mean post-treatment time is indicated (*A* 5-FU, *B* Fluoronucleotides, *C* FUPA, *D* FBal, *E* Reference signal – fluorobenzene)

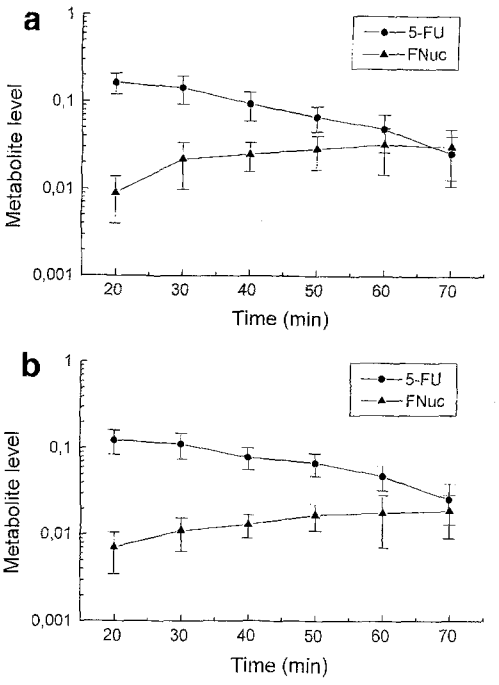

Fig. 3a, b Peak areas obtained for 5-FU and FNuc in **a** the CSM colon carcinoma and **b** the hypopharynx carcinoma following an i.v. bolus injection of 130 mg kg^{-1} 5-FU. The data represent the results of summed spectra as described in Materials and methods. For each 20-min acquisition, the mean post-treatment time is indicated. Results represent mean values ± SE for 14 (CSM colon carcinoma) and 12 (hypopharynx carcinoma) tumours, respectively. Peak areas are expressed in arbitrary units (percentage of the fluorobenzene reference signal)

shown). MTX pre-treatment had no significant influence on the measured 5-FU catabolism. In contrast, when thymidine was applied, no FBal was detectable in the CSM tumour, and in the hypopharynx carcinoma the maximal level of the FBal/5-FU_0 ratio was significantly lower than that noted after treatment with 5-FU alone (0.36 ± 0.15 versus 0.09 ± 0.08).

Concerning kinetics, the decay of 5-FU fitted well to a mono-exponential function in both tumours and all treatment modalities, thus allowing determination of the drug's half-life (Table 3). It was calculated from the original spectra without application of the moving average method. It increased significantly when thymidine was coinjected (Table 3).

Considering the standard deviation of the growth delay shown in Table 1 and the FNuc/Ref ratio illustrated in Fig. 3, it becomes obvious that there was a remarkable variation with respect to both parameters in the colon and hypopharynx carcinoma xenografts investigated. To evaluate whether the individual tumour regression correlated with the individual FNuc/Ref ratio, these data of both xenografts were plotted for single-agent treatment with 5-FU (Fig. 5a, b). The correlation was significant in both cases (hypopharynxcarcinoma: $r = 0.52$, $P = 0.041$; CSM colon carcinoma: $r = 0.59$, $P = 0.013$).

Table 2 Average FNuc/5-FU ratio and number of animals transplanted with the hypopharynx carcinoma (Hyp) or the CSM colon carcinoma and treated with either 5-FU (130 mg/kg), 5-FU + methotrexate (100 mg/kg), or 5-FU + thymidine (400 mg/kg). The standard deviation is given in parentheses. For calculation of the FNuc/5-FU_0 ratio, the initial 5-FU concentration and the plateau level recorded for FNuc after 50–60 min were used

	FNuc/5-FU_0 (Hyp)	*n* (Hyp)	FNuc/5-FU_0 (CSM)	*n* (CSM)
5-FU	0.13 (0.068)	12	0.24 (0.11)	14
5-FU/methotrexate	0.22 (0.084)*	6	0.38 (0.105)*	5
5-FU/thymidine	0.18 (0.086)	5	0.36 (0.125)*	6

* $P < 0.05$ as compared with treatment with 5-FU alone

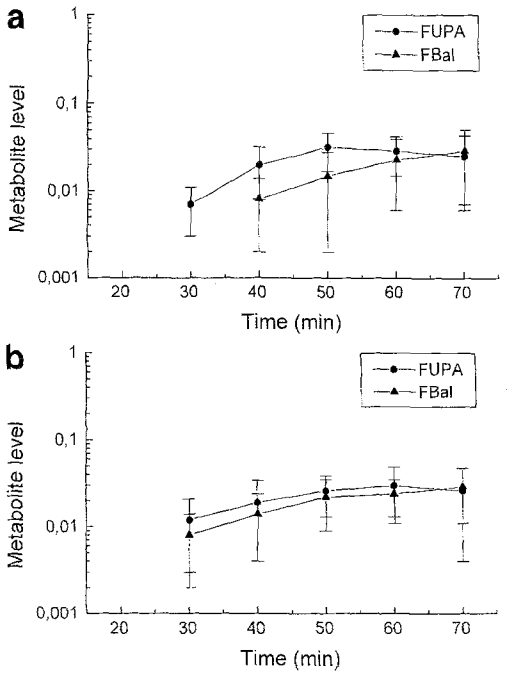

Fig. 4a, b Peak areas obtained for FUPA and FBal in **a** the CSM colon carcinoma and **b** the hypopharynx carcinoma following an i.v. bolus injection of 130 mg kg^{-1} 5-FU. Results represent mean values ± SE for 14 (CSM colon carcinoma) and 12 (hypopharynx carcinoma) tumours, respectively. Peak areas are expressed in arbitrary units (percentage of the fluorobenzene reference signal)

Discussion

Treatment of the hypopharynx carcinoma with 100 mg kg^{-1} 5-FU caused significantly less growth retardation than did treatment of the CSM colon carcinoma ($P < 0.001$; Table 1). This effect is apparently caused by the higher level of FNuc in the CSM tumour as compared with the hypopharynx carcinoma (0.045 ± 0.016 versus 0.017 ± 0.004 FNuc/Ref ratio, $P < 0.001$; Figs. 3a, 4a), The higher level of FNuc in the CSM tumour is due to the better 5-FU uptake of the latter as compared with the hypopharynx carcinoma (0.165 ± 0.048 versus 0.124 ± 0.045 5-FU$_0$/Ref ratio, $P < 0.05$; Figs. 3a, 4a) and to the higher efficiency of the conversion of 5-FU to FNuc in the CSM tumour (0.24 ± 0.11 versus 0.13 ± 0.068 FNuc/5-FU$_0$ ratio, $P < 0.01$; Table 2).

Table 3 Kinetics of the disappearance of 5-FU and number of animals transplanted with the hypopharynx carcinoma (Hyp) or the CSM colon carcinoma and treated with either 5-FU (130 mg/kg), 5-FU + methotrexate (100 mg/kg), or 5-FU + thymidine (400 mg/kg). The standard deviation is given in parentheses

	$t_{1/2}$ (Hyp, min)	n (Hyp)	$t_{1/2}$ (CSM, min)	n (CSM)
5-FU	28.2 (11.2)	12	25.5 (9.9)	14
5-FU/methotrexate	29 (6.7)	6	32.6 (12.3)	5
5-FU/thymidine	40.5 (9.3)*	5	44.6 (14.3)*	6

* $P < 0.05$ as compared with treatment with 5-FU alone

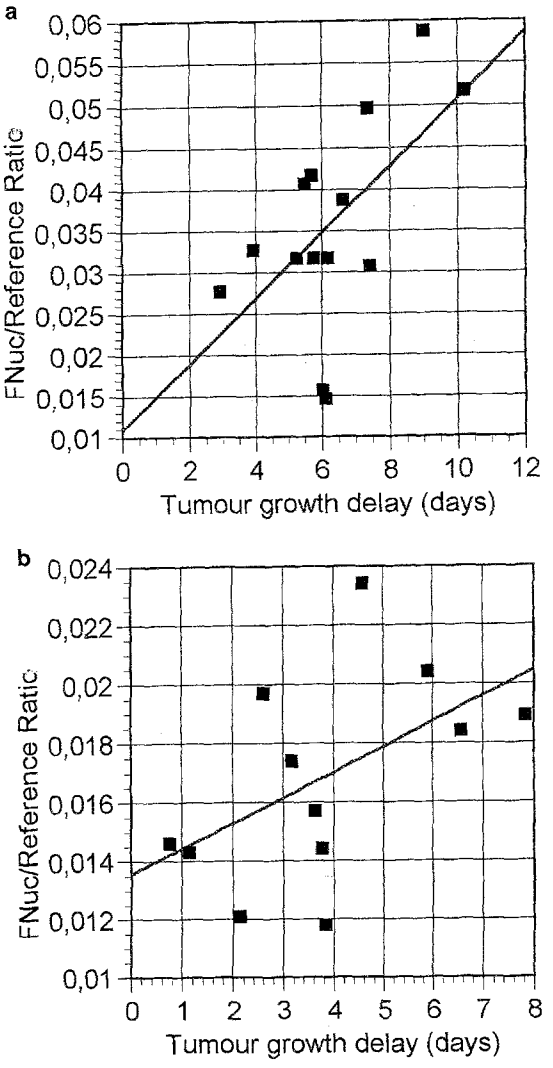

Fig. 5a, b Linear regression plots of the correlations between the tumour FNuc/Ref ratio detected at 50–60 min after administration of 130 mg kg^{-1} 5-FU and the growth delay observed in **a** the CSM colon carcinoma ($r = 0.59$, $P = 0.013$) and **b** the hypopharynx carcinoma xenografts ($r = 0.52$, $P = 0.041$)

MTX and thymidine-induced interactions with 5-FU can result in enhanced or diminished 5-FU cytotoxicity [2, 6]. In our study, the CSM colon carcinoma treated with the MTX/5-FU or thymidine/5-FU schedule actually showed a synergistic action for both drugs, whereas this was not the case in the hypopharynx carcinoma (Table 1). However, the FNuc/5-FU$_0$ ratio was increased in both tumours, this increase not being significant in the hypopharynx carcinoma treated with the thymidine/5-FU schedule (Table 2). Therefore, in the hypopharynx carcinoma the FNuc/5-FU$_0$ ratio did not serve as a predictor for the treatment efficacy of the combined treatment modality as compared with treatment with 5-FU alone. On the basis of the data obtained in this study, it was not possible to decide on the reason for this finding. However, one has to take into account three circumstances:

1. Both thymidine and MTX increase the level of FUTP and, thus, the FNuc/5-FU_0 ratio, the former doing so by prolonging the plasma half-life of 5-FU by competitively inhibiting dihydrouracil dehydrogenase [12, 25] and the latter, by increasing the concentration of PRPP [1].
2. While increasing the RNA-directed cytotoxicity [6, 21], both drugs diminish the DNA-directed toxicity, MTX doing so by depleting the cells of reduced folates [3, 24] and thymidine, by opening the salvage pathway of deoxynucleotide biosynthesis [2].
3. FdUMP is present in much lower concentrations than FUTP and thus contributes to a much lower extent than FUTP to the FNUC peak. In cases in which it is bound to thymidylate synthetase, it is invisible to NMR [7].
Thus, in tumours for which DNA-directed cytotoxicity is more important than RNA-directed toxicity, the combined therapy schedule may lead to reduced cytotoxicity even though the level of NMR-measurable FNuc is increased.

When MTX was injected before 5-FU, the efficiency of the conversion of 5-FU to FNuc increased significantly by 58% in the CSM tumour and by 69% in the hypopharynx carcinoma as compared with treatment with 5-FU alone (Table 2). However, this increase was rather low as compared with the results of other studies using the CD8F1 murine mammary tumour and the WK tumour [11, 16], where the FNuc level increased by a factor of 2–3. Several mechanisms may be responsible for this low increase in 5-FU conversion to FNuc in the xenografts investigated in this study, such as improper scheduling or a low tumour growth rate. However, an interval of 15 h between the application of MTX and that of 5-FU should be sufficient for the build-up of PRPP [1]. On the other hand, the presence of a low growth fraction in a tumour population results in a low increase in PRPP [4] because MTX is an S-phase-specific drug. The tumours investigated in our study have tumour-doubling times of 5 and 8 days, respectively, as compared with 24 h for the WK tumour. Accordingly, they are fairly unresponsive to MTX as compared with the WK tumour, which was used by McSheehy et al. [16].

Naguib et al. [17] found increased dihydrouracil dehydrogenase activity in solid tumours as compared with their mother tissue. Thus, the detection of FUPA and FBal in the hypopharynx and CSM tumours by ^{19}F-NMR spectroscopy is probably due to catabolism of 5-FU in the tumours. In the present study, MTX did not have any detectable effect on the catabolism of 5-FU. This has previously been described by Hull et al. [9] in a study on patients receiving an MTX/5-FU schedule. In contrast, pre-treatment with thymidine prolonged the half-life of 5-FU (Table 3), slowed down the formation of FUPA and led to a tremendous reduction in the formation of FBal. Grem [8] has pointed out that this is due to a reduction in the dihydrouracil dehydrogenase-mediated conversion of 5-FU to FUH_2 and an inhibition of the ureidopropionase-mediated conversion of FUPA to FBal. In contrast to Sijens and Ng [19], we did not detect FUH_2 in the human carcinoma xenografts investigated in this study. However, after combined administration of thymidine and 5-FU, the aforementioned investigators detected FUH_2 in the livers of mice, where the concentration of FUH_2 should be higher than in tumour tissues.

We have found that early changes in phosphorus metabolism measured by ^{31}P-NMR spectroscopy in the hypopharynx carcinoma xenograft are of predictive value for individual tumour regression after chemotherapy with cisplatin [23]. For 5-FU chemotherapy, several other investigators [14–16, 19, 20] have confirmed that the ^{19}F-NMR-measurable FNuc formed in tumours after drug application are pertinent for predicting drug cytotoxicity. This hypothesis is validated by the results of the present study, especially the significant linear correlation between the tumour FNuc/Ref ratio detected by ^{19}F-NMR spectroscopy and the volume decrease induced by a dose of 130 mg kg^{-1} 5-FU in the hypopharynx carcinoma ($r = 0.52$, $P = 0.041$) and the CSM colon carcinoma ($r = 0.59$, $P = 0.013$; Fig. 5a, b).

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