Prediction of 5-fluorouracil cytotoxicity towards the Walker carcinosarcoma using peak integrals of fluoronucleotides measured by MRS in vivo

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Summary 19F-magnetic resonance spectroscopy (MRS) can be used to non-invasively monitor metabolism of 5-fluorouracil (5FU) to cytotoxic fluoronucleotides (FNuct). We investigated whether the levels of FNuct formed from 5FU and observed in vivo by MRS in the Walker carcinosarcoma predicted cytotoxicity. Fifty mg kg\textsuperscript{-1} 5FU caused tumour FNuct formation and, when repeated daily for 1 week, significant tumour growth inhibition (P<5%). Twenty-five mg kg\textsuperscript{-1} 5FU produced less tumour FNuct (P<5%) and did not cause significant tumour regression. Tumour regression and tumour FNuct formation were also suppressed by 50 mg kg\textsuperscript{-1} 5FU combined with a molar equivalent dose of allopurinol (P<2%). Tumour extracts were analysed by hplc and MRS confirming the observations in vivo and demonstrating that peak integrals in vivo were directly proportional to FNuct and FNuct concentrations. Hplc analysis of extracts showed that 50% of FNuct in tumours treated with 5FU was the cytotoxic nucleotide FUTP; this was lower to 5% by a molar equivalent dose of allopurinol (P<2%). Twenty-five mg kg\textsuperscript{-1} 5FU also produced significantly less FUTP (36%) than the 50 mg kg\textsuperscript{-1} dose (P<5%). These results suggest that MRS-detectable changes in tumour FNuct (mostly in FUTP) can be used to predict 5FU cytotoxicity.

The xenobiotic 5-fluorouracil (5FU), which has been in clinical use for the past 30 years, is now used predominantly against gastrointestinal and breast cancer (Heidelberger et al., 1983). Clinical success is low against human tumours, even in combination with other drugs such as methotrexate or allopurinol, while host toxicity remains a problem (Mackintosh & Tattersall, 1987). However, until more effective drugs are found it is necessary to improve the efficacy of those drugs currently available.

Chemotherapy could be optimised more easily if early indicators of tumour responsiveness were available. 19F-NMR spectroscopy (MRS) is a non-invasive technique that can be used to follow the transformations of a drug as they occur in situ (Stevens et al., 1984). The metabolism of 5FU is summarised in Figure 1. Detoxification (catabolism) occurs predominantly in the liver, while cytotoxicity is most likely mediated by the formation of two fluoronucleotides: 5-fluoro-2'-deoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). FdUTP can be incorporated into DNA but is thought to be removed by uracil-DNA glycosylase and thus this deoxynucleotide may not be important in 5FU cytotoxicity (Pinedo et al., 1988) (Figure 1). FdUMP inhibits thymidylate synthase which in the absence of thymidine blocks DNA synthesis, while the ribonucleotide interferes with RNA metabolism (Heidelberger et al., 1983). FUTP and FdUMP have different chemical shifts in vitro (0.1–0.2 p.p.m. resolution) (Keniry et al., 1986; Cabanac et al., 1988), but these and the other fluoronucleotides are indistinguishable by MRS in vivo, having similar pH-dependent chemical shifts of 4.5–5 p.p.m. downfield from 5FU. We will therefore refer to the combined peak as fluoronucleotide (FNuct). This FNuct signal, which would also include signals from any FUDP-sugars can be resolved by MRS in vivo from the two 5-fluoronucleotides (FUrd and FdUrd) which have a chemical shift of 3.5–3.8 p.p.m. downfield from 5FU (Keniry et al., 1986; Malet-Martino et al., 1986).

FNuct has been observed in mouse liver in vivo by MRS (Hull et al., 1987; Prior et al., 1987; Cabanac et al., 1988). More intense signals were detected in mouse tumours (Stevens et al., 1984; Hull et al., 1986; Koutcher et al., 1987) and in a rat pituitary tumour, the SG prolactinoma (Griffiths et al., 1987). Apparent increases in the amount of FNuct generated in vivo can be induced by appropriate combination chemotherapy, but this was not related to increased cell death or tumour regression (Stevens et al., 1984; Hull et al., 1987; Koutcher et al., 1987). Such work is gradually being extended to patients where signals corresponding to 5FU and the catabolite 5-fluoro-β-alanine (FBal) (19 p.p.m. upfield from 5FU) have been detected in liver (Wolf et al., 1987). It was reported recently that FNuct has been observed in liver metastases after treatment with 5FU (Semmler et al., 1988). However, these experiments did not demonstrate whether the presence of the FNuct peak was related to cytotoxicity.

We have studied 5FU metabolism in the Walker carcinosarcoma grown s.c. in rats which rapidly metabolises 5FU to FNuct (Klubes et al., 1978). Our objective was to

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determine whether MRS-visible levels of this species predicted cytotoxicity. We aimed to alter tumour levels of FNuc in vivo in two ways by: (a) varying the 5FU dose from 25 mg kg⁻¹ (low) up to 50 mg kg⁻¹ and 120 mg kg⁻¹ (high); (b) modulating 5FU metabolism by combination chemotherapy using allopurinol (HPP). HPP is activated to oxypurinol monophosphate which inhibits the enzyme orotidylate decarboxylase responsible for the conversion of orotidine monophosphate to uridine monophosphate. The inhibition raises intracellular levels of orotate which has a higher affinity for the enzyme pyrimidine phosphoribosyl transferase (PPRT) responsible for the conversion of 5FU to 5-flourouridine monophosphate (FUMP) (Schwartz & Handschumacher, 1979) (Figure 1). The activation of HPP also uses the co-substrate phosphoribosylpyrophosphate which is used to convert 5FU to FUMP and thus HPP indirectly inhibits the PPRT pathway by two mechanisms which should lead to an overall reduction in FNuc (Howell et al., 1981). Indeed using MRS in vivo, we have shown that HPP causes a significant decrease in FNuc formation from 5FU in isolated Walker 256 and Ehrlich ascites tumour cells (Prior et al., 1987).

Materials and methods

Drugs and chemicals

5FU as the sodium salt in water was obtained from David Bull Laboratories (Warwick, UK). 5-Fluorotryptophan, FdUMP and HPP were purchased from Sigma Chemical Co. (Poole, Dorset, UK). HPP (4-hydroxypyrazolo pyrimidine) (or allopurinol) was dissolved in 0.9% NaCl containing 0.2 m NaOH. 5-Fluorouridine monophosphate (FUMP) was purchased from Calbiochem (Cambridge, UK).

Tumour management

In all studies the rats used were female Wistar of weight 180–200 g. The Walker 256 carcinosarcoma cells (passage number 550–553) were stored at 10⁸ cells ml⁻¹ in Hams F10 medium containing 20 mM HEPEs and 5% fetal calf serum (FCS) (Flow Laboratories) with 10% dimethyl sulphoxide at −196°C. Cells were thawed, washed twice in 0.9% saline and injected i.p. (5 × 10⁵) into the rats. After 4–5 days the cells were harvested, washed and viability was determined using the trypan blue (0.1%) exclusion test. The cells were suspended in F10 medium containing 5% FCS and were injected s.c. (2 × 10⁵) into one flank; one injection was given to each rat. Tumours were normally grown for 6–10 days and were used for MRS studies when greater than 400 mm², i.e. a diameter greater than that of the surface coil used. Assuming the tumours were prolate spheroids with a density of 1.0, this area would correspond to a weight of 4 g (Klubes et al., 1978). Walker carcinosarcomas of this size and up to weights of about 15 g show little or no necrosis (Stubbbs et al., 1988).

For studies of inhibition of tumour growth the rats (9 or 10 in each group) were injected s.c. with Walker cells on day 0. On the next and each subsequent day up to and including day 7, rats received 0.9% saline i.p., 50 mg kg⁻¹ or 25 mg kg⁻¹ 5FU i.p. or the combination of 5FU and a molar equivalent dose of HPP (52 or 26 mg kg⁻¹) i.p. Rats were weighed each day to assess toxicity. On day 8 the rats were killed and the tumours were excised and weighed.

¹⁹F-NMR spectroscopy

All spectra were obtained at 75.5 MHz and 20–22°C using a 1.9T 30 cm horizontal bore magnet (Oxford Research Systems). Spectra (480 transients, spectral width of 3–4 kHz) were obtained in vivo using a 1.5 cm diameter 2-turn surface coil, with 14 μs radiofrequency pulses and a 1 s repetition time. With this coil a 90° flip angle at the coil centre corresponded to a 7 μs pulse. The receiver gains and all other acquisition parameters were identical for each tumour studied. Rats were anaesthetised with an i.p. injection of sodium pentobarbitone before the jugular vein was cannulated. The surface coil was positioned so that it was on the middle of the tumour and touching the surface. In this position we obtained shims from the proton peak of 32 ± 9 Hz for controls and 35 ± 10 Hz for tests (mean ± s.d., n = 8). Rats without any previous treatment received 25, 50 or 120 mg kg⁻¹ 5FU as an i.v. bolus injection (controls), or in addition a molar equivalent i.v. bolus of HPP, 15 min before the 5FU. Simultaneously with the administration of 5FU, individual spectra were acquired for consecutive 8 min intervals until 67 min at which time the tumours were rapidly excised and freeze-clamped. The spectra shown in the figures are the sums of four consecutive 8 min spectra for the labelled time periods. The time points for the kinetic time course plots indicate the midpoint of each total time period. This technique is equivalent to a 4-point smoothing procedure for 8 min data, but it was necessary in order to achieve reasonable signal-to-noise at doses below 120 mg kg⁻¹ 5FU, and we also used this method to compare the different 5FU doses. These spectra, obtained in vivo, were given 19 Hz line broadening and the integrals of the peaks were measured using the Oxford Research Systems analysis program to give an area (in arbitrary units) for each peak. In Figure 8, where arbitrary units are plotted against nmol g⁻¹, the zero point on the ordinate does not necessarily correspond to 0 nmol g⁻¹, but is the sensitivity limit of MRS under our conditions. All chemical shifts are referenced relative to 5FU (0 ppm).

Acid extracts

Tumour extracts were made (normally 3 g from each tissue) using 4 ml of cold 6% (v/v) perchloric acid per gram of tissue and were neutralised with KOH. The extracts were freeze-dried and concentrated to a 3 ml solution. Aliquots (100 μl) were stored at –20°C for hplc and the remainder was analysed by MRS. In vitro spectra (at least 1,500 transients with a spectral width of 6 kHz) were obtained using a solenoid coil, 9 μs pulses (flip angle=90°) and an 8.5 s repetition time. For quantitative analysis a capillary containing 300 nmol of 5-fluorotryptophan solution was used as an external standard. The same amount of standard was used for every extract and was always placed in the same position in the coil. An 8.5 s interval was found to be sufficient to relax 5FU, FBal, FNuc and the external standard under our conditions in vitro.

Hplc

Samples were analysed using a 100 mm × 5 mm Hypersil-APSiI weak anion-exchange column and a Jones Chromatography Integrator (Hengoed, Glamorgan, UK). A 16 min linear concentration gradient was generated from potassium phosphate (3 g l⁻¹), pH 2.8 (Aristar grade, BDH chemicals, Poole, UK) and potassium phosphate (90 g l⁻¹), pH 3.3 both prepared with doubly glass-distilled water and Analar HCl. All samples added to the column contained 0.05 mM cytidine diphosphate as an internal standard. Measurements were made at both 254 nm and 280 nm to quantify the fluorinatedUMP, UDP and UTP according to the method of M.J.W. Prior (personal communication). FUMP, FDUMP, FUDP and FUTP were used as standards, the latter two synthesised from FUMP by the method of Pogolotti et al. (1981).

Statistics

Most analyses used an unpaired two-tailed Student's t test, assuming equal variances. Where variances were significantly different (F test, 2.5 percentage points) an approximate t test was used. In Table 1, where three groups were compared, Gabriel's one-way analysis of variance was used (Kendall & Stuart, 1968).
Results

The Walker carcinosarcoma grew in a reproducible manner doubling in size approximately every 24 h, and normally reached a weight of about 6 g after 8 days' growth (Table I). This growth was not inhibited by a daily dose of 25 mg kg\(^{-1}\) 5FU and at this dose general toxicity was low. Two separate studies (one shown) demonstrated that doubling the daily 5FU dose caused a significant reduction in tumour weight by day 8, although this was associated with a large weight loss for the rat (Table I). Coadministration of HPP prevented toxicity, but the combination regime did not cause a significant reduction in tumour weight. This result is similar to observations made using murine leukaemia cell lines in vitro, where HPP antagonised the growth inhibitory action of 5FU (Schwartz & Handschumacher, 1979).

Following the i.v. bolus injection, low doses of 5FU (which had no effect on the tumour growth) produced very little FNuct signal; indeed FNuct was seen in only two out of four injected with this dose. With a 50 mg kg\(^{-1}\) dose (which did cause significant retardation of tumour growth) 5FU was seen in all the tumours within 8 min. The fluoronucleotides (FNuct) gradually appeared while the 5FU peak integral diminished until 33-67 min, when the peak area ratio of FNuct/5FU was about one. The pattern of metabolism that we observed in the absence of HPP was consistent from one tumour to another. Figure 2 includes a summary of results from three tumours that received this dose. In acute experiments with a dose of 120 mg kg\(^{-1}\) (too toxic to be used for studies on tumour growth) the pattern of 5FU metabolism was similar (Figure 3). The apparent lag-phase (0-17 min) at this dose may not be real since zero arbitrary units does not necessarily reflect 0 nmol g\(^{-1}\) but just the sensitivity limits of the machine (see Materials and methods).

Figure 4 shows this typical pattern of metabolism in an individual tumour that received the high dose of 5FU.

An injection of a molar equivalent dose of HPP, 15 min before 5FU, markedly altered the concentration of 5FU metabolites. FNuct was not seen at all at 50 mg kg\(^{-1}\) (Figure 5) and only small signals were seen in two of four tumours at the 120 mg kg\(^{-1}\) dose (Figure 6). The rate of 5FU disappearance also appeared slower at 50 mg kg\(^{-1}\) (Figure 2), while at 120 mg kg\(^{-1}\) the 5FU signal remained at the same intensity throughout the experiments (Figure 3).

Signals corresponding to the catabolite FBal were seen only occasionally in vivo and were apparently independent of the 5FU dose or drug combination used. Signals corresponding to the 5-fluorounucleosides (FNucs) were never observed in these tumours in vivo. The chemical shift between SFU and FNuct showed some small intertumoral variation, but on average suggested a pH\(_i\) range of 6.9-7.3 using the titration curves of Keniry et al. (1986). In summary, the above results showed that a higher tumour formation of FNuct (increased FNuct peak integral) was associated with increased 5FU cytotoxicity towards the Walker carcinosarcoma.

Extracts from these tumours were analysed quantitatively by both MRS and hplc. MRS in vitro confirmed observations in vivo, namely the presence of 5FU and FNuct and the absence of the FNucs metabolites in the tumours (Figure 7). Spectra in vitro demonstrated that FBal was nearly always present following 5FU administration, but overall there was no correlation between FBal concentration and the type of drug regime given to the rat. Table II compares quantitation of the tumour extracts by MRS in vitro with the mean peak areas measured in vivo. This table

### Table I

| Treatment | Tumour wt (g) | % wt increase of rat | Tumour wt (g) | % wt increase of rat |
|-----------|---------------|----------------------|---------------|----------------------|
| None      | 6.8 ± 1.5     | 11.8 ± 0.2           | 6.4 ± 2.2     | 12.5 ± 3.0           |
| 5FU       | 5.6 ± 1.0     | 4.7 ± 1.0            | 1.2 ± 0.5\(^a\) | -11.9 ± 6.5          |
| 5FU + HPP | 7.0 ± 1.0     | 4.6 ± 1.1            | 2.8 ± 0.7     | 9.7 ± 16.2           |

Measurements were made 8 days after inoculation of the rats with tumour cells. Animals were treated daily for 7 days as described in Methods. Results are from two separate studies and are the mean ± s.e. of 10 rats per group (25 mg kg\(^{-1}\) 5FU) and 9 rats per group (50 mg kg\(^{-1}\) 5FU).

\(^{a}\)P < 0.05, when compared to no treatment using Gabriel's one-way analysis of variance.

Figure 2 Peak integrals of 5FU and FNuct in the Walker carcinosarcoma following an i.v. bolus injection of 5FU (50 mg kg\(^{-1}\)) in the absence and presence of HPP. Rats receiving a molar equivalent dose of HPP were injected i.v. 15 min before 5FU. Results are the mean ± s.e. of three tumours (control) and four tumours (HPP). ○, 5FU in controls; ●, FNuct in controls; △, 5FU in HPP-pretreated rats.

Figure 3 Peak integrals of 5FU and FNuct in the Walker carcinosarcoma following an i.v. bolus injection of 5FU (120 mg kg\(^{-1}\)) in the absence and presence of HPP. Rats receiving a molar equivalent dose of HPP were injected i.v. 15 min before 5FU. Results are the mean ± s.e. of 5 tumours (control) and 4 tumours (HPP). ○, 5FU in controls; ●, FNuct in controls; △, 5FU in HPP-pretreated rats.
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Peaks

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FU,

5-fluouracil;

FN, 5-fluoronucleotides

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Figure

4 Metabolism

by

Walker
carcinosarcoma

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Rats

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Each

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processed

with

19Hz

line

broadening.

Peaks

identified

as

follows:

FU,

5-fluorouracil;

FN, 5-fluoronucleotides

deoxy

and

dideoxy)

and

FUDP-sugars.

Figure

5 Metabolism

by

Walker
carcinosarcoma

of

5FU

(50mg/kg

-1) in

the

presence

of

HPP.

Rats

received

an

i.v.

bolus

injection

of

5FU

at

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Figure

6 Metabolism

by

Walker
carcinosarcoma

of

5FU

(120mg/kg

-1) in

the

presence

of

HPP.

Rats

received

an

i.v.

bolus

injection

of

5FU

at

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FNuct, i.e. an 86% and 63% reduction in FNuct concentrations following 50mg kg-1 and 120mg kg-1 5FU doses, respectively. In vivo, the alteration of 5FU metabolism caused by HPP, which affected both the FNuct and 5FU peaks, was most evident as a highly significant decrease in the FNuct/5FU ratio at both of the 5FU doses (Table II).

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(Table II), probably because the measurements were effectively taken at different times. The peak integral in vivo was determined from a mean time of 50min and the extracts were made at 67min, immediately after the MRS accumulation was complete. Figures 2 and 3 show that the FNuct levels at both 5FU doses were still increasing at 50min and the 5FU levels were decreasing. The effect of using these two different time points would also explain why the two slopes in Figure 8 are significantly different (P<0.1%): a peak area of 2,000 represents 100nmol g-1 for 5FU, but 240nmol g-1 for FNuct. Thus the 5FU measurement in vivo (area measured at 33–67 min) would be overestimated and the FNuct

Figure 7 Spectra of extracts from Walker carcinosarcoma freeze-clamped 67min after a bolus injection of 5FU (120mg kg-1). a, 5FU alone; b, 5FU 15min after a molar equivalent dose of 5HPP. The spectral width was 6kHz. Each spectrum was from 1,500 transients, with an 8.5s repetition time and was processed with 19Hz line broadening. Peaks identified as follows: FT, 300nmol 5-fluorotryptophan; FU, 5-fluouracil; FN, 5-fluoronucleotides (deoxy and non-deoxy) and FUDP-sugars, FBAL, α-fluoro-β-alanine.

Figure 8 Peak integrals in vivo, vs. concentrations of 5FU and FNuct measured in extracts from the Walker carcinosarcoma. Data were used from both the in vivo (peak integral) and in vitro (quantified concentration) values that applied to each individual tumour. Results are from tumours that received both 50 and 120mg kg-1 5FU doses in the presence (tests) and absence (controls) of HPP. △, 5FU (controls); ◀, 5FU (tests); ●, FNuct. Slopes were generated using linear correlation, r (5FU)=0.96; r (FNuct)=0.89.
Table II: Comparison of measurements made by MRS in vivo and in vitro of the levels of 5FU and FNuct in the Walker carcinosarcoma following treatment with 5FU with or without HPP pretreatment.

| Treatment | 5FU (nmol g⁻¹) | FNuct (nmol g⁻¹) | FNUct/FU ratio | 5FU (arbitrary units) | FNuct (arbitrary units) | FNuct/FU ratio |
|-----------|----------------|------------------|----------------|-----------------------|------------------------|---------------|
| SFU (25 mg kg⁻¹) | 0 | 63 ± 21 | 23 ± 15 | | 278 ± 194 | 296 ± 250 | 1.0 ± 0.9 |
| SFU (50 mg kg⁻¹) | 24 ± 24 | 172 ± 35 | 22 ± 12 | 7.2 | | | |
| SFU + HPP (50 mg kg⁻¹) | 74 ± 28 | 24 ± 24 | 39 ± 30 | 0.3 ± 0.3 | | | |
| SFU (120 mg kg⁻¹) | 202 ± 32 | 273 ± 28 | 78 ± 19 | 1.4 ± 0.2 | | | |
| SFU + HPP (120 mg kg⁻¹) | 494 ± 26 | 101 ± 39 | 25 ± 4 | 0.2 ± 0.1 | | | |

Samples are the same as in Figures 2 and 3. Results are mean ± s.e. where (n) is the number of tumours. The times of the spectra were as follows: in vivo, 50 min (33–67 min); in vitro, 67 min.

This data show that lowering the 5FU dose resulted in significantly lower intracellular concentrations of FUPT, but that the drug combination with HPP caused a much larger reduction in FUPT of 94% and 91% for 50 and 120 mg kg⁻¹ respectively. There were fairly small differences, however, in the levels of FUPT following treatment with 5FU and HPP, compared with 5FU alone (Table III). This is surprising since the locus of HPP action is indirect inhibition of formation of FUMP from 5FU leading to reduced concentrations for FUPT and FUPT (Schwartz & Handschumacher, 1979). It suggests that the conversion of FUMP to FUPT by nucleoside monophosphate kinase has become rate-limiting, perhaps because of competition from other monophosphates, such as orotidine monophosphate, that are increased following HPP metabolism.

Discussion

5FU is activated by intracellular enzymes to 5-fluorouracil (FUMP), two of which (FUPT and FUDP) are cytotoxic (Heidelberger et al., 1983). In principle MRS could be used to observe FNuct formation non-invasively and thus could be a potential indicator of tumour responsiveness. In practice, however, FUDP or the ternary complex of FUDP, thymidylate synthase and the co-factor 5,10-methylenetetrahydrofolate are formed at cytotoxic concentrations that are below the sensitivity of MRS. The ternary complex has been reported to be NMR-visible in...
human pancreatic adenocarcinoma cells but this result was not reproducible and required high doses of 5-fluoro-2'-deoxyuridine in vitro (Malet-Martino et al., 1986). Intracellularly, FdUMP can reach concentrations up to 5 nmol g⁻¹, which would be sufficient to inhibit thymidylate synthase completely (Berne et al., 1987; Washtein, 1984; Klubes et al., 1978), but which is still below MR sensitivity in vivo. In any case, the levels of free intracellular FdUMP may not correlate with cytotoxicity. For example, the Walker carcinosarcoma formed 0.4 nmol g⁻¹ FdUMP, four times the concentration synthesised by the 5FU sensitive L1210 cells, but remained relatively insensitive to a single dose of 5FU since it cleared FdUMP very rapidly (Klubes et al., 1978). Similarly, HPP caused a 60% reduction of FdUMP levels in rat colon carcinoma, but did not affect the amount of thymidylate synthase inhibition (Berne et al., 1987). Also, despite thymidylate synthase inhibition, DNA synthesis can continue through salvage of extracellular thymidine (Heidelberger et al., 1983).

The mechanism of 5FU cytotoxicity via FUTP remains less clear. FUTP becomes incorporated into RNA, interfering with RNA methylation, tRNA formation and protein synthesis (Heidelberger et al., 1983). RNAs may be altered and lead to a slow release of 5FU over many hours (Spears et al., 1984). Increases in the intracellular concentration of FUTP are associated with increased cell killing in a number of cell lines (Schwartz & Handschumacher, 1979; Cadman et al., 1981; Benz & Cadman, 1981), and FUTP may reach concentrations up to 50 times those of FdUMP (Cadman et al., 1981). These observations suggest not only that FUTP alone would be MRS-visible, but also that changes in its concentration in tumours could be used to predict cytotoxicity.

We have shown using MRS in vivo that FNuct was rapidly formed in the Walker carcinosarcoma following a single 5FU dose at and above 50 mg kg⁻¹. Analysis of the extracts from these tumours by MRS in vitro and by hplc demonstrated that lowering the 5FU dose to 25 mg kg⁻¹, or using combination chemotherapy with HPP, decreased the intracellular concentration of FNuct. hplc showed that 50% of this FNuct in controls was the cytotoxic species FUTP. Compared to the 50 mg kg⁻¹ dose of 5FU, the low dose of 5FU produced 64% less intracellular FUTP, while co-administration of HPP caused a 90% reduction in FUTP levels. These large changes in FNuct which can be measured quantitatively in vitro at a single time-point (in this case 67 min) may be detected in vivo, as a highly significant change in the FNuct/5FU peak integral ratio. Furthermore, under the conditions used in these experiments, the levels of FNuct and 5FU formed in vivo could be estimated since the peak integrals were shown to be proportional to the final concentrations measured in the extracts at 67 min. Drug regimes that produced a mean FNuct peak integral below 300 arbitrary units (from Figure 8 this would be < 35 nmol g⁻¹) were associated with reduced cytotoxicity since they did not cause significant inhibition of tumour growth. Included in these drug regimes was the 5FU (50 mg kg⁻¹) + HPP combination which produced less FUTP than the 5FU (25 mg kg⁻¹) alone (Table III), even though the former regime seemed more cytotoxic (Table I). Since the locus of HPP action is to inhibit the formation of FUMP this might encourage increased metabolism of 5FU towards FdUMP if the levels of deoxyribose-1-phosphate are sufficiently high (Figure 1), leading to a higher proportion of DNA-directed cytotoxicity in the presence of HPP. However, as discussed above, this mode of cytotoxicity may not be significant in the Walker carcinosarcoma (Klubes et al., 1978), and it could be that at these lower concentrations FUTP is rapidly incorporated into RNA where it is undetectable by our methods.

Using MRS in vivo, different levels of FNuct formation have been reported in murine tumours that have differential sensitivity to 5FU (Hull et al., 1986). In this case FNuct formation was observed in the ‘5FU-resistant’ M5076 cells but was absent in the ‘5FU-sensitive’ sarcoma 180 cells. However, in this experiment the S180 tumours were highly necrotic and the M5076 cells were mostly viable and later results with viable tumours suggested a higher anabolite/catabolite ratio for S180 compared to M5076 (W. Hull, personal communication).

5FU was immediately and clearly visible in tumours at both of the high doses of 5FU. This signal declined at a faster rate than the FNuct signal increased probably because 5FU underwent catabolism and efflux from the tissue as well as anabolism. However, in the presence of HPP the disappearance of 5FU from tumours was slower. HPP may reduce hepatic clearance of 5FU in patients (Howell et al., 1981) and these results suggest an additional effect on tumour catabolism. Signals corresponding to FNucs were never observed by MRS in vivo or in vitro (detection limit in vitro was 10 nmol g⁻¹) and could not be distinguished from other nucleotides on our ion-exchange hplc. Of the possible catabolic products of 5FU metabolism only FBal was seen in vivo, but not consistently. Analysis of extracts showed that in fact FBal was present at all doses of 5FU at concentrations ranging from a mean of 20 up to 80 nmol g⁻¹, suggesting that MRS resolution in vivo was limited by the breadth of the signal and/or the effect of saturation due to a long T₂ for FBal in vivo. Yet in the extracts there was no correlation between FBal concentrations and the concentrations of FNuct or 5FU. It is possible the FBal we observe owes its source to the rapid 5FU catabolism that occurs in rat liver (Cabanac et al., 1988) and it may be extracellular. So, neither FBal peaks in vivo, nor FBal concentrations in vitro, are related to 5FU cytotoxicity. These observations are similar to that made by Hull et al. (1988), who did not find a correlation between clinical success and the pharmacokinetics of 5FU catabolism in patients receiving 5FU or 5FU plus methotrexate chemotherapy.

The use of peak integrals to estimate the concentration of drugs in vivo may only be appropriate in our experimental protocol and probably could not universally be applied. It does suggest, however, that at higher fields and with the development of quantitative methods, MRS could be used as a predictive tool by the clinician. It is also pertinent to note that we have not determined whether multiple treatments of the same animal would result in the 5FU metabolic profiles we have described here. Nevertheless, our study of the MRS signals observed in the Walker carcinosarcoma following an injection of 5FU or HPP and 5FU, shows that only the FNuct peak integral predicted which drug regime was likely to induce tumour regression. To our knowledge this is the first demonstration that the size of the FNuct peak is relevant to determining the cytotoxicity of 5FU towards tumours. Our results suggest that MRS in vivo could assess the sensitivity of tumours that are responsive to 5FU via the cytotoxic species FUTP. Furthermore, it has been shown that the mechanism of action of 5FU can be directed towards FUTP and RNA cytotoxicity by using methotrexate (Benz & Cadman, 1981) or thymidine (Takimoto et al., 1987). It is therefore possible that MRS could be used to determine the optimal scheduling of this type of combination chemotherapy. These possibilities are currently under investigation.

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