Influence of pH on the uptake of 5-fluorouracil into isolated tumour cells

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Summary To investigate the possible dependence of 5-fluorouracil (5FU) uptake in tumours on the intra- (pHi) and extracellular (pHe) pH, a pH gradient (ΔpH) was imposed across the plasma membrane of ascites tumour cells in vitro, similar to that known to occur in some solid tumours in vivo, by incubation in media of pHi 5-6. A > 2:1 (intracellular/extracellular) accumulation of radio-labelled 5FU occurred after 5 min incubation of the cells with 0.5 mm 5FU at pHe of 5.0, 5.5 or 6.0. 5FU metabolism is slow under these conditions, and 5FU uptake was not affected by longer incubations up to 20 min, nor by the absence of a sodium gradient. pHi was estimated from the distribution of the weak acid, 5,5-dimethyl-2,4-oxazolidione ([14C]DMO) across the cell membrane. There was significant correlation between the intracellular/extracellular 5FU ratio and pHi (from pHi 6-8), ΔpH and pHi (P < 0.02). Similar results were obtained with HT29 cells. Incubation with a drug that made plasma membranes permeable to H+ significantly decreased 5FU uptake in Lette cells. The co-transport of 5FU may occur on a proton symport using the proton motive force of the ΔpH.

Keywords: 5-fluorouracil; intracellular pH; extracellular pH; ascites tumour cells; pH gradient

5-Fluorouracil (5FU) has been in use as an anti-cancer agent for more than three decades since its synthesis in 1957 by Heidelberger et al. Nowadays, it is predominately used to treat solid tumours such as colon and breast cancers and occasionally for head and neck, and lung cancers, and adjuvant treatments of potential micrometastases, either alone or in combination with other drugs such as interferon, methotrexate or leucovorin. 5FU may be anabolized to cytotoxic nucleotides or deactivated by catabolism, an event occurring predominantly in the liver, but also in other tissues and some tumours (Naguib et al, 1985). 5FU metabolism has been extensively studied by 19F magnetic resonance spectroscopy (MRS) in cultured cells, and in vivo in tumour-bearing animals and patients (for review see Findlay and Leach, 1994). 5FU cytotoxicity is caused by irreversible inhibition of thymidylate synthase (TS) via the generation of the anabolite 5-fluoro-2-deoxyuridine monophosphate (FdUMP) leading to an inhibition of DNA synthesis, and by incorporation of 5-fluorouridine-5-triphosphate (FUTP) into RNA (FU-RNA). The degree of sensitivity to either, or both, of these cytotoxic nucleotides is tissue dependent (Heidelberger et al, 1983). TS inhibition tends to be favoured by prolonged exposure of low 5FU concentrations (approximately 15 μM) for several days, whereas FU-RNA formation tends to be favoured by brief exposures to high concentrations (1 mm) for a few hours (see Aschele et al, 1992). The intracellular concentrations of 5FU and its metabolites are also dependent on the transport and uptake of 5FU into the target cells (Heidelberger et al, 1983).

19F-MRS studies have shown that 5FU appears to be retained longer in tumours than in normal tissues (Presant et al, 1990; Guerquin-Kern et al, 1991; Findlay et al, 1993). Enhanced retention has also been shown to be significantly associated with response (Presant et al, 1990; Findlay et al, 1993), perhaps because higher concentrations of 5FU will sustain its anti-tumour effects by favouring the lasting presence of toxic metabolites at target tissue sites (Peters et al, 1993). The elimination half-lives (t½) of 5FU in the VX2 tumour of the rabbit (Wolf et al, 1990), and the Walker 256 adenocarcinoma of the rat (El-Tahtawy and Wolf, 1991), were found to be about 1 h (63-73.2 and 42.2-59.4 min respectively), and greatly exceed the t½ of 5FU in rat plasma (Au et al, 1983), which is similar to that reported in humans (5-15 min) (Cohen et al, 1982). Presant et al (1994) found that the response of patients to 5FU could be predicted from the rate of loss of tumour 5FU signal, measured by 19F-MRS. These observations suggest that elucidation of the mechanisms by which 5FU accumulates in tumours could be of clinical significance as they may aid the development of more rational combination chemotherapy.

The pH of tumours measured non-invasively with 31P-MRS has been shown, in most circumstances, to represent intracellular pH (pHi) (Stubbs et al, 1992), whereas microelectrode measurements mainly sample extracellular pH (pHe) (for review see Vaupel et al, 1989). Overall, MRS measurements show that tumours and normal tissues have close to neutral pHi values (6.9-7.4) (Griffiths, 1991), whereas pHi values for tumours are more acidic than pHe (by about 0.3-0.5 pH units). pHi values for normal tissues are on the alkaline side of pHe with a range from 7.2 to 7.6. Thus, the pH gradient across the membrane of normal cells is from (relatively) acid intracellularly to alkaline extracellularly. In contrast, in tumours the pH gradient is negative, i.e. the reverse of that in control tissues. The acidic pHe in tumours is probably due to the high glycolytic rate associated with tumours and the subsequent extrusion of cellular acids.

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A $^{14}$F-MRS study by Guerquen-Kern et al (1991) showed that the $t_{1/2}$ of 5FU elimination was 2.5-fold longer at pH 6.9 than at pH 7.3 in a rat fibrosarcoma. A glucose-induced reduction can lead to an even greater decrease in pH $\overline{3}$ (see Gerweck et al, 1991), thus causing an increase in the negative pH gradient, and it is possible, therefore, that the observed trapping of 5FU in tumours is related to the difference between pH $\overline{3}$ and pH $\overline{4}$ across the tumour cell membrane. The aim of the present study was to try to relate the distribution of radiolabelled 5FU to the distribution of H+ (referred to as the pH gradient; $\Delta$H) across the cell membrane of Lettre cells (a line of mouse Ehrlich ascites cells), and human HT29 adenocarcinoma cells. This was carried out by varying pH $\overline{4}$ values and assessing the induced pH $\overline{3}$ from the distribution of the labelled weak acid, 5,5-dimethyl-2,4-oxazolidione (DMO) across the membrane of the cells. Cells were incubated with 5FU at concentrations of 0.1–0.5 mm, which were in the range of peak plasma concentrations measured in mice (Hill and Bibby, 1994) and patients in the clinic (Findlay et al, 1996), and would not be rate limiting for transport (Yamamoto et al, 1981; Heidelberger et al, 1983).

**MATERIALS AND METHODS**

**Drugs and chemicals**

5FU was obtained as the sodium salt in water from David Bull Laboratories (Warwick, UK). 5FU-6-3H (spec. act. 20 Ci mmol$^{-1}$) was purchased from Sigma Chemical Company (Poole, Dorset, UK) and $[^14]$CDMO from Amersham International (Amersham, UK). HP/$\beta$ scintillant fluid was obtained from Beckman, and oil (2:1 of di-n butyl phthalate and dinonyl phthalate) from BDH chemicals, Poole, UK, and carbonyl cyanide $p$-(trifluoromethoxy)phenyldihydrazone (FCCP) from Sigma.

**Production of Lettre cells**

White Swiss TO male mice weighing approximately 30 g were injected intraperitoneally with 0.1 ml of 10$^6$ cells ml$^{-1}$ that grew as ascites. After 7–10 days, the cells were removed from the peritoneal cavity and suspended in Heps-buffered saline (HBS) containing approximately 100 $\mu$l of heparin. The suspension was centrifuged and the pellet was resuspended in isotonic saline solution to 30% v/v (c. 3 x 10$^6$ cells ml$^{-1}$, i.e. 300 mg of cells). All experiments were conducted at a room temperature of 20–22°C.

**Measurement of pH$\overline{3}$ using the $[^14]$CDMO distribution method and $[^3]$H5FU distribution across cell plasma membranes**

Suspensions of Lettre cells (6 x 10$^7$ cells ml$^{-1}$), which is the standard for this type of experiment (Bashford, 1994), were incubated with $[^3]$H5FU (6 $\mu$Ci), $[^14]$CDMO (2 $\mu$Ci) and 0.1 mm or 0.5 mm cold 5FU, in duplicate, in each of a range of McIlwain buffer solutions (from pH 5.0 to 8.0), the final concentration of the buffer being 20 mm (Dawson et al, 1969) for 5, 10 and 20 min. The final volume was 1 ml made up with isotonic saline solution. In some experiments the incubation medium was modified by the addition of 10 mm glucose, or the replacement of K+ for Na+. The pH$\overline{3}$ were measured on a pH meter immediately before the experiment. After incubation, 0.3 ml of cell suspension was layered over 0.1 ml of oil and centrifuged for 10 s revealing three layers: pelleted cells, an oil layer and the incubation medium (for details see Bashford et al, 1983). A sample of supernatant (60 $\mu$l) was counted in a scintillation counter. The oil (middle layer) was aspirated, and the pellet (bottom layer) was resuspended in 1 ml of lithium nitrate (0.1 m lithium nitrate, 0.003% Triton X-100, Na+ and K+ free) and dispersed by sonication. An aliquot (200 $\mu$l) of the pellet suspension was counted in a liquid scintillation counter. Na+ and K+ ions were measured on 0.1 ml of the pellet suspension using lithium nitrate as carrier, on the assumption that total Na+ plus K+ in the pellet could be used as an indicator of pellet water.

**Calculation of intracellular pH from DMO distribution**

The pH$\overline{3}$ was calculated from the equation (Bashford 1994):

$$\text{pH}_{\overline{3}} = \log_{10} \left\{ \frac{D_m}{D_{ext}} \left(10^{\text{[KDMO}]} + 10^{\text{[NaNO}} \right) - 10^{\text{[NaNO}} \right\}$$

where $D_m$ = (pellet c.p.m. $\times F$) / [Na+ + K+]; $F$ = molarity of Na+ plus K+ in cell water and dilution factor; $D_{ext}$ = supernatant c.p.m./aliquot size in µl; $D_m/D_{ext}$ = ratio of DMO concentration inside/outside the cells; and $pK_{DMO} = 6.3$.

The pH gradient ($\Delta$H) = pH$\overline{4}$ – pH$\overline{3}$.

**Experiments using carbonyl cyanide $p$-(trifluoromethoxy)phenyldihydrazone (FCCP)**

FCCP is an ionophore, which depolarizes plasma membranes making them permeable to H+. Experiments were set up as described above, except for the addition of FCCP (final concentration 10 µm) or the ethanol vehicle (0.1%) to triplicate samples at pH$\overline{3}$ of 5, 6, 7 and 8. In half of the experiments, triplicate samples ± FCCP were also incubated for 5 min, before centrifugation (10 s), removal of the supernatant, and cold perchloric acid (4%) extraction of the pellet for ATP spectrophotometric analysis as described in Bergmeyer (1974).

**HT29 cells**

The human HT29 adenocarcinoma cells were grown in McCoy’s medium containing 10% fetal calf serum (FCS) in a 5% carbon dioxide atmosphere at 37°C. After trypsinization using a salt solution of 5% trypsin and 2% EDTA and washing in an isotonic salt solution, cells were resuspended in the McIlwain buffers described above and were used for experiments at a concentration of 10$^6$ cells ml$^{-1}$. All experiments were conducted at room temperature.

**Statistics**

Analysis (5FU$_{int}$/5FU$_{ext}$ ratio vs pH$\overline{3}$, pH$\overline{4}$, or pH$\overline{5}$) involved the correlation coefficient ($r$) significance test where the correlation between the two measurements is significant at the 5% level. $P$ = probability, n = sample size and the degrees of freedom = n–2. A further assessment of the differences in the degree of correlation of these three parameters with the 5FU$_{int}$/5FU$_{ext}$ ratio was made using an analysis of variance (ANOVA), which is the recommended test when comparing differences between three or more values. Differences in the 5FU$_{int}$/5FU$_{ext}$ ratio ± FCCP were tested using a paired t-test.
RESULTS

Dependence of 5FU distribution on pH

The time-dependent uptake by isolated Lettre cells incubated at room temperature with radiolabelled 5FU is shown in Figure 1. It demonstrates (a) the accumulation of intracellular 5FU (approximately 0.3 mm) and (b) that 5FU equilibrated across the cell membrane within 5 min, as longer incubations of up to 20 min did not significantly increase the amount of radioactivity in the pellet. This suggested that little or no metabolism of 5FU occurred in this time interval. Indeed, Yamamoto and Kawasaki (1981), showed that 5FU metabolism was negligible in Ehrlich ascites cells even after 10 min incubation in medium containing glucose at 37°C, and we have also shown that incubation of Lettre cells with 2 mM 5FU at 37°C for 90 min resulted in only c. 5% 5FU metabolism (McSheehy et al., 1991). Thus, 5FU metabolism in Lettre cells is slow, even in the presence of nutrients, and therefore the distribution of 5FU across the cell membrane after 5 min incubation represented essentially a steady state.

Similar to the radiolabelled 5FU distribution, the incubation time (5, 10 or 20 min) made no difference to [3H]DMO distribution in either the pellet or the supernatant fraction (results not shown). This suggested that equilibrium of [3H]DMO along the pH gradient was also reached within the 5-min incubation period, and all subsequent experiments were performed at 5 min. Note that a negative ΔpH (–ΔpH) demonstrates that the pHi was more acid than the pHj.

Figure 2 shows the relationship between pHj and the ΔpH in one experiment, and how the distribution of [3H]5FU across the plasma membrane, expressed as the 5FUint/5FUext ratio, relates to the ΔpH. The results in Figure 2A show that the more acid the extracellular pH, the larger the –ΔpH was across the cell membranes, i.e. the greater the difference from zero. Over the pH range of 5–8 there was a significant correlation (P < 0.001) and this was also unaffected by the incubation time (results not shown). The results in Figure 2B suggested that when the ΔpH became more negative (i.e. the –ΔpH increased), there was a trend towards a higher 5FUint/5FUext ratio. The maximum 5FUint/5FUext ratio in this experiment, approximately 2.1, was observed at a ΔpH gradient of –1. At more negative values of ΔpH, there was no further increase in the 5FUint/5FUext ratio.

The mean data from several experiments in which the 5FUint/5FUext ratio was compared with pHj, pHi and the ΔpH at an extracellular 5FU concentration of 0.5 mM are shown in Figure 3. A point of inflection occurs in the graph of 5FUint/5FUext vs pHj at or above a pHj of 6.0 (Figure 3A). From pHj 6–8 there is a highly significant negative linear correlation (P = 0.002) with the 5FUint/5FUext ratio (i.e. the ratio rises as pHj falls), but no further increase in 5FUint/5FUext occurs below pHj 6.0. This results in a threefold decrease in the 5FUint/5FUext ratio from pHj 6 to 8. When the 5FUint/5FUext ratio was plotted against pHj, a similar inflection was observed at c. pHj 6.8 (Figure 3B), and c. –0.8 for the graph of 5FUint/5FUext vs ΔpH (Figure 3C). Again, the linear parts of the graphs in Figure 3B and C showed significant correlation coefficients (P = 0.017 and P < 0.001 for pHj and ΔpH respectively). Similar correlations were seen at an extracellular 5FU concentration of 0.1 mM (results not shown). At 0.5 mM 5FU, the mean ± s.d. correlation coefficients in these experiments for the plot of 5FUint/5FUext vs pH were 0.96 ± 0.03, 0.98 ± 0.04 and 0.88 ± 0.03 for pHj, ΔpH and pHi, respectively, and an ANOVA demonstrated that pHj and ΔpH were significantly better correlated than pHi with the 5FUint/5FUext ratio (P = 0.0003). It should be noted that pHi is not an independent variable as the measurement is dependent on the pHj value (see equation in Materials and methods).
Alteration of constituents of the incubation medium

Two experiments were performed in which glucose (10 mM) was included in the incubation medium to cause intracellular acidification from glycolytically produced lactic acid. The 5FU concentration in the extracellular medium was 0.5 mM. Glucose decreased the uptake of DMO: the DMO_{int}/DMO_{ext} ratio was about 2 with glucose and 3.5 without glucose, implying that intracellular acidification had taken place after glucose was added to the medium, since from the equation on page 6 pH_i is proportional to DMO_{int}/DMO_{ext}. A representative example of the 5FU results is shown in Figure 4, in which the 5FU_{int}/5FU_{ext} ratio is plotted against pH_i and pH_e. The 5FU_{int}/5FU_{ext} ratio did not rise to such a high plateau at acidic pH_i, the highest values in the two experiments were 1.6–1.7. Nevertheless, there was still an approximately linear fall in the 5FU_{int}/5FU_{ext} ratio over the pH range 6–8 (Figure 4A), whereas the pH_i values were clearly shifted to lower values in the presence of glucose (Figure 4B) resulting in a decrease of the −ΔpH (Figure 4C).

The importance of the Na^+ gradient in this process was investigated by replacing Na^+ in the incubation medium with K^+. When the cells were incubated in the presence of 0.5 mM 5FU, there was no decrease in the maximum 5FU_{int}/5FU_{ext} ratio, nor in the pH gradient when two parallel experiments were compared in which Na^+ was present in the medium (results not shown).

Effect of FCCP

FCCP depolarizes the plasma membrane making it permeable to H^+ and uncouples oxidative phosphorylation. The effect on 5FU uptake over the pH_i range 5–8 is shown in Figure 5. The addition of 10 μM FCCP during the 5-min incubation reduced by 35% the 5FU_{int}/5FU_{ext} ratio at pH 5, 6 and 7. In addition, there was a small
but significant decrease in the pHi of 0.12 at pH 6 and 7, while both the intra- and extracellular [Na+] and [K+] were unchanged by FCCP treatment at all pHi values. In four of these eight experiments, the total intracellular [ATP] was determined to be 1.0 ± 0.1 μmol g⁻¹ (mean ± sem), and FCCP caused a mean decrease in this concentration of 33%.

Distribution of 5FU across HT29 colon adenocarcinoma plasma cell membranes

Similar results to those found in the Lettrec cells were also found when human HT29 cells were incubated with 5FU (Figure 6). Using the same pHi range of 5–8, the maximum recorded ΔpH was −1.5 (at pH 5), resulting in a mean 5FUᵢᵦ/5FUᵢₑ ratio of 1.66. This ratio was significantly different (P < 0.05) from that measured when the ΔpH was close to zero (pH 7 or 7.5). When the 5FUᵢᵦ/5FUᵢₑ ratio was plotted against pHᵢ, there was a highly significant negative correlation over the pHᵢ range 6–8 (P = 0.0003), similar to that found for Lettre cells.

DISCUSSION

We have studied the pH dependence of 5FU uptake in two tumour cell lines: the human HT29, which has been used in ¹⁹F-MRS pharmacokinetic studies in vivo (McSheehy et al., 1997), and murine Lettre cells in which many plasma membrane studies have been performed (Bashford and Pasternak, 1984). Variation of the pH of the suspending medium (pHᵢ) was used to alter ΔpH, i.e. the difference between pHᵢ and pHᵦ, so that a −ΔpH of 1.5–2.0 could be attained at pHᵢ 5. An increase in the −ΔpH correlated with an increase in the 5FUᵢᵦ/5FUᵢₑ ratio, i.e. uptake of 5FU by the cells. This 5FU uptake by Lettre cells was markedly inhibited in the presence of the ionophore FCCP.

How can the relationship between 5FU uptake and pH be explained? In the absence of significant metabolism, the most obvious explanation would be that the drug is distributed across the cell membrane as a weak acid. The enolic hydroxyl groups of 5FU ionize as acids, but with a pKᵢₐ of 8.1. If 5FU distributed like a weak acid, little intracellular accumulation would be expected under physiological conditions, because pKᵢₐ > pHᵢ. However, in

![Figure 5](image-url)  
**Figure 5** Effect of FCCP on the 5FUᵢᵦ/5FUᵢₑ ratio in Lettre cells. Results show the mean ± sem of eight experiments using 0.5 mm 5FU over the pHᵢ range of 5–8 where ● is control and □ 10 μM FCCP. **P < 0.01, *P < 0.05** compared with FCCP-treated (paired t-test).

![Figure 6](image-url)  
**Figure 6** Relationship between ΔpH across HT29 cell plasma membranes and (A) pHᵢ and 5FUᵢᵦ/5FUᵢₑ. Results show the mean ± sem from four experiments incubating 10⁶ cells ml⁻¹ for 5 min with 0.1 mm 5FU, where pH gradient (ΔpH) = pHᵦ − pHᵢ, the presence of 0.5 mm 5FU at a pHᵦ of 6.0 and pHᵦ of 6.7 (where the 5FUᵢᵦ/5FUᵢₑ of ≥2:1 was measured in the Lettre cells – see Figure 3), the calculated ratio would be about 1.0:1. At pHᵦ 7.31 and pHᵦ 6.63, which are the pH values more typical of physiological conditions, the calculated ratio would still only be 1.15:1. Thus, our observations cannot be explained simply on the basis that 5FU distributes as a weak acid.

Wohluheuer et al. (1980) showed that transport of 5FU into Novikoff rat hepatoma cells was inhibited by uracil, suggesting that 5FU uptake involved facilitated transport via the uracil transporter. They also showed that the initial rate of transport was dependent on pH and related to pKᵢₐ, as only the uncharged species is transported. The pKᵢₐ of uracil is 9.2, so it will be almost entirely in the uncharged form at all reasonable physiological pH values. 5FU, despite having a lower pKᵢₐ of 8.1, would be 90–95% in the uncharged form at around pHᵦ 6.5–7.0 (the most likely pHᵦ values to be encountered in tumours), whereas at a pHᵦ of 6.0, 99% would be uncharged. In both cases, near-maximum initial rates of transport will be allowed, whereas at a pHᵦ 8.0, 50% of the 5FU would be charged and the initial rate of transport would be markedly reduced. These considerations do not explain the 'steady-state' 5FU distribution observed in the present experiments. This is because the
uracil transporter will transport the uncharged form of its ‘ligand’ equally efficiently either into or out of the cell, the amount of 5FU transported in either direction depending on the concentration of uncharged 5FU available on the inside and outside of the cell membrane. In our experiments, 5FU retention only occurs at pH values (intracellular or extracellular) in the range of 6–7, when the concentration of the uncharged species would not be significantly different from one side of the plasma membrane to the other. In addition, Wohlhueter’s work investigated initial rates of 5FU transport, whereas our experiments study an equilibrium, or steady state, that is unchanged for up to 20 min after incubation (Figure 1).

Yamamoto and Kawasaki (1981) studied facilitative transport in Ehrlich ascites cells (the cell line from which Lettre cells were derived) suspended in 5 mM glucose, and showed that 5FU uptake was directly coupled to the ATP derived from glycolysis. This active transport occurred in the absence of a Na+ gradient. The initial rate was slightly stimulated by Na+ (1.3-fold), suggesting that Na+ here was acting as a co-factor and not in creating an electrochemical potential, as occurs in the active transport of glucose and many amino acids. They concluded that 5FU uptake in these cells resembled the ATP-driven, Na+-independent amino acid transport that occurs in Escherichia coli. We also found that the SFU_in/SFU_ex ratio was unaffected by the absence of Na+ in the incubation medium, suggesting that in the steady state it was Na+ independent.

An alternative explanation is that the electrochemical concentration gradient of H+ that we have imposed across the plasma membrane, provides a proton motive force (p.m.f.) which can be used to perform transport work. The p.m.f. (or Δp) is related to the ΔpH:

\[ \Delta p = \Delta \Psi - (Z \Delta \Phi) \]

where \( Z \) is a constant of 59 mV at 37°C and \( \Delta \Psi \) is the plasma membrane potential (Harrison and Lunt, 1980). Co-transport of 5FU may occur by proton symport, as long as sufficient p.m.f. exists, i.e. there continues to be a −ΔpH or the ΔpH is not depolarized. This hypothesis predicts that there would be no intracellular accumulation of 5FU in the absence of a −ΔpH across the cell membrane, or when the plasma membrane is depolarized. Figure 3C (Lettre cells) and Figure 6B (HT29 cells) show that the SFU_in/SFU_ex ratio is indeed found to be 1.0 when the ΔpH across the cell membrane is zero, and, similarly, the SFU_in/SFU_ex ratio is <1.0 when the cell membrane ΔpH becomes positive (i.e. \( pH_i \) is more alkaline than \( pHi \)). Table 1 shows the calculated p.m.f. for Lettre cells over the \( pHi \) range studied. Note that p.m.f. decreases by approximately two-fold from \( pHi \) 5 to \( pHi \) 8, which is a similar change in magnitude to that measured for the SFU_in/SFU_ex ratio.

The hypothesis was tested experimentally by incubating Lettre cells with FCCP. FCCP is an ionophore which depolarizes plasma membranes, making them permeable to H+, and uncouples mitochondrial oxidative phosphorylation. Under the conditions of our study, FCCP significantly decreased the SFU_in/SFU_ex ratio by 35% at \( pHi \) 5–7, and the total intracellular [ATP] by a similar amount. As the ion gradients were unchanged (as assessed by the distribution of K+ and Na+ across the plasma cell membrane), we have hypothesized that the [ATP] was still sufficient to maintain ion gradients, and that the depolarization of the plasma membrane was responsible for decreasing the SFU_in/SFU_ex ratio. This is consistent with the idea that 5FU uptake is dependent on p.m.f. However, these experiments are in no way conclusive evidence of our hypothesis, and further investigations are being pursued to distinguish between the depolarizing and uncoupling effects of FCCP.

| pHi | ΔpH | ΔH (mV) | p.m.f. | SFU_in/SFU_ex ratio |
|-----|-----|--------|-------|---------------------|
| 5   | −1.66 | −4     | 99    | 1.97                |
| 5.5 | −1.23 | −15.5  | 86    | 2                   |
| 6   | −0.76 | −27    | 70    | 1.96                |
| 6.5 | −0.44 | −38.5  | 64    | 1.62                |
| 7   | 0.06  | −50    | 47    | 1.13                |
| 7.5 | 0.05  | −61.5  | 59    | 0.95                |
| 8   | 0.29  | −73    | 57    | 0.71                |

Values for pHi, ΔpH and the SFU ratio (FU_in/FU_ex) are taken from the values in Figure 3 (mean of five experiments), while ΔΨ is calculated from the data in Bashford and Pasternak (1984) in which for these cells, the ΔΨ depolarizes by 23 mV for each unit decrease in the pH. When pH > 7.3, p.m.f. (Δp) is calculated from the equation: \( \Delta p = \Delta \Psi - Z \Delta \Phi \), in which Z is a combination of constants equal to 59 mV.

Our results and hypothesis are consistent with the MRS observations of Guerquin-Kern et al. (1991), who showed that more 5FU was retained in a rat fibrosarcoma at lower pHi (< 6.9), induced by glucose infusion, than at pH 7.3, similar to the findings made in this study. Furthermore, Gerweck et al. (1991) found that in tumours administration of glucose to the host induced a decrease in pHi but caused an even greater decrease in pHi 60 min later, thus increasing the −ΔpH still further. Thus, although pHi was not measured in the study of Guerquin-Kern et al. (1991), it is possible that the increased retention of 5FU was due to an increased −ΔpH across the tumour cell membranes. The results of our experiments in which glucose was added to the medium, reported in Figure 4, are broadly in agreement with our hypothesis. Glucose lowered the pHi, and thus decreased the −ΔpH (Figure 4C) and consequently the SFU_in/SFU_ex ratio.

Prerequisite for 5FU cytotoxicity is intracellular metabolism of the prodrug to 5-fluorouracil, and thus the first step necessary for anti-tumour activity is 5FU uptake by the target cells. Furthermore, the therapeutic ratio will be enhanced if uptake into normal cells is minimized. Our results suggest ways in which both of these favourable effects occur with 5FU. At a lower pHi, in which the −ΔpH is large, such as occurs in solid tumours, there would be an enhancement of 5FU uptake, whereas normal cells (in which ΔpH is usually positive) will tend to exclude 5FU. In solid tumours this could lead to an accumulation of 5FU relative to other normal tissues, which is precisely what has been recorded in biopsies (Peters et al, 1993) and also by non-invasive 1H MRS in animal models and in the clinic (Findlay et al, 1993; Presant et al, 1994). The hypothesis suggests potential means of manipulating the tumour pH environment for therapeutic gain. For example, one of the many drugs used in combination with 5FU in the clinic is interferon α, which has been shown in vitro to increase activity of the plasma membrane Na+/H+ antiporter in human tumour cells leading to an increase in pHi (Maheshwari et al, 1991). Indeed, we have recently shown in HT29 tumours xenografted in nude mice that interferon α increased the −ΔpH via an increase in pHi and increased 5FU retention in these tumours (McSheehy et al, 1997).

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