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Gene expression predicts differential capecitabine metabolism, impacting on both pharmacokinetics and antitumour activity

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ABSTRACT

Capecitabine is converted into 5′-deoxy-5-fluorocytidine (5′DFCR), 5′-deoxy-5-fluorouridine (5′DFUR) and 5-fluorouracil (5-FU) by CES1 and 2, CDD, and TP, in both liver and tumour. 5-FU is catabolised by DPD. Gene expression analysis of these enzymes was undertaken in fresh human hepatocytes, mouse liver, colorectal cancer cell lines and xenografts. Cell lines with low CDD expression (<1.5) had 5′DFCR/5′DFUR cytotoxicity ratios >2 and cell lines with TP/DPD < 0.6 had 5′DFUR IC_{50} > 50 μM (SRB assay). A pharmacokinetic/pharmacodynamic study in nude mice bearing HCT 116 xenografts and treated with capecitabine by oral gavage assessed pharmacokinetic, gene expression and antitumour activity. Low liver CDD correlated with high 5′DFCR plasma concentrations in mice. CDD expression was ~100-fold higher in fresh human hepatocytes than mouse liver, explaining the higher plasma 5′DFUR concentrations reported previously in humans. Tumour 5-FU concentration correlated with TP/DPD and with tumour response. These studies identify the potential utility of gene expression analysis and drug monitoring in tumour in patients.

1. Introduction

Capecitabine (Xeloda®; Hoffmann La Roche) is an oral fluoropyrimidine which mimics a continuous infusion of 5-fluorouracil (5-FU).[1–3] Capecitabine is metabolised in the liver and tumour by carboxylesterases (CES1 and CES2)[4] and cytidine deaminase (CDD) to 5′-deoxy-5-fluorocytidine (5′DFCR) and 5′-deoxy-5-fluorouridine (5′DFUR), respectively. 5′DFUR is subsequently activated by pyrimidine phosphorylases (thymidine phosphorylase (TP) and possibly uridine phosphorylase (UP)) by metabolism to 5-FU.[5,6] Dihydropyrimidine dehydrogenase (DPD), highly expressed in liver, metabolises 5-FU to dihydro 5-FU, inactivating it. In tumour cells, 5-FU interacts with its pharmacological target, thymidylate synthase (TS).

During capecitabine development, a physiologically-based pre-clinical model was built to predict the pharmacokinetics of capecitabine in patients.[7] This complex mathematical model was based on enzymatic activities of capecitabine metabolising enzymes determined *in vitro* in mouse tissues, limited pharmacokinetic data over 24 h after the administration of a single dose of capecitabine and physiological parameters (blood flow rate, volume of individual organs) based on the literature data. The model was shown to predict tumour exposure to 5-FU and integrated the interspecies differences between humans and mice. However, the population

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pharmacokinetics studies carried out in patients including pharmacodynamic data (toxicity) did not show any clear relationship between systemic metabolite exposure in patients and toxicity, suggesting that drug and metabolite concentrations in plasma do not accurately reflect the tissues' exposure to the drug.[8,9] In another study, drug concentrations and enzyme activity in tumour, liver and plasma were determined in patients treated with capecitabine.[10] The study confirmed high concentrations of 5-FU in tumour tissue, but failed to establish a correlation between drug concentrations and enzyme activity.

Pre-clinical models using human cancer xenografts can address these issues. Due to the extensive metabolism of capecitabine, the levels of expression of the enzymes involved in its activation, both in liver and tumour, are likely to play a role in its antitumour effect. Indeed, overexpression of CDD in the T24 human bladder cancer cell line sensitised cells to 5′DFCR.[11] In xenograft tumours with high TP expression, higher tumour 5-FU concentrations were demonstrated after the administration of capecitabine than 5-FU suggesting tumour-specific activation of capecitabine by TP.[12–14] However, *in vitro,* similar 5′DFUR- and 5-FU-mediated cytotoxicity were observed in COLO320 cells (TP negative) and in WiDr cells (high TP), suggesting that UP might also metabolise 5′DFUR in the absence of TP.[15] Finally, to date, the expression of TS in tumours is the best predictive marker of response to 5-FU systemic therapy, but this has not been demonstrated for capecitabine.[16]

To develop a comprehensive view of the contribution of each enzyme to the metabolism of capecitabine, an *in vitro* study using 6 colon cancer cell lines was combined with a gene expression analysis of capecitabine-metabolising enzymes to identify key parameters for optimal cytotoxicity. A similar approach was also used *in vivo* in nude mice bearing the human colon cancer xenograft HCT 116. Capecitabine-metabolising enzymes gene expression was determined in xenograft tissue and mouse liver over a 3-week treatment period, using capecitabine at 2 dose levels. A similar gene expression analysis was carried out in human hepatocytes to identify interspecies differences in drug metabolism. Capecitabine and metabolite concentrations were also determined in liver, plasma and tumour xenografts.

2. Materials and methods

2.1. Cell lines and human hepatocytes

HCT 116, HCT8, HCT15, HT29, SW620 and COLO205 human colon cancer cells were obtained from the European Collection of Cell Cultures ECACC (Salisbury, UK). Fresh human hepatocytes from eight individual donors were obtained from the UK Human Tissue bank (Leicester, UK) after ethical approval from NHS Lothian (LREC/2003/8/42). Cells were received approximately 8 h after isolation. Only hepatocytes with viability greater than 80%, as determined by trypan blue exclusion at the time of collection, were used in the study. At the time of arrival, cells were centrifuged, resuspended in Tri-reagent® and stored at −80 °C until RNA extraction.

2.2. Gene expression analysis of capecitabine-metabolising enzymes by qRT-PCR

For cell lines, cells were collected in Tri-reagent® (SIGMA). For xenografts and liver samples, tissues were homogenised in Tri-reagent® (SIGMA) using a Mikro dismembrator (Sartorius, Epsom UK). Total RNA was prepared according to manufacturer's instructions followed by DNAse-treatment with Turbo-Free® DNAse (Ambion, Huntingdon UK). The quality of RNA was evaluated by electrophoretic analysis on a 2100 Agilent Bioanalyser. All qRT-PCR experiments were carried out with RNA integrity number >9. RNA concentrations were determined using a Nanodrop ND-1000. The transcripts of interest were amplified according to a previously published method.[17] Results were normalised by β-2-microglobulin (B2M) expression in both mouse liver and fresh hepatocytes, and by RNA polymerase II large subunit (POLR2) in HCT 116 xenografts (HCT 116 has a very similar expression for B2M and POLR2) and colon cancer cell lines. To compare gene expression in mouse and human tissues, a conversion factor was calculated between mouse and human standards used for quantification after verifying that all reaction efficiencies were close to 100% for all genes investigated. Three livers and six xenograft samples were analysed per time-point in each group unless the amount of RNA recovered from the tissue was insufficient for qRT-PCR. Gene expression is expressed as the ratios between the gene of interest (GOI) and the reference gene (REF).

2.3. In vitro cytotoxicity of capecitabine, 5′DFCR, 5′DFUR and 5-FU in human colon cancer cell lines

Drug concentrations that inhibited 50% of cell growth (IC_{50}) were determined using the sulforhodamine B technique.[18] Cells were plated on day 1 in 96-well plates at a density of 2500 cells/well for HCT 116, 3500 cells/well for HCT8 and HT29, 5000 cells/well for HCT15, 6000 cells/well for SW620 and 7000 cells/wells for COLO205 in a volume of 150 μl/well. All cell lines were treated on day 2 with increasing concentrations of capecitabine (0.1–10 mM), 5′DFCR (10 nM–100 μM), 5′DFUR (2.5–500 μM) or 5-FU (0.5–250 μM) for 24 h. After drug exposure, cells were washed once with cold PBS and placed in 200 μl of drug-free medium for 72 h after the end of drug exposure. The cells were then fixed with trichloroacetic acid and stained with sulforhodamine B. Optical densities were measured at 540 nm with a Biohit BP-800 (Bio-Hit, Helsinki, Finland). The results are based on three independent experiments performed in triplicate.

2.4. Pharmacokinetic/pharmacodynamic studies

Six-week-old C57/Bl6 Nu/Nu mice were obtained from Cancer Research UK (London, UK) and quarantined for 2 weeks before the start of experiments. Animal experiments were carried out under a project licence issued by the UK Home Office, and UKCCCR guidelines[19] were followed rigorously.

Bilateral HCT 116 xenografts were obtained by subcutaneous injection of 10^7 cells/flank. Animals bearing HCT 116 xenografts were treated with vehicle or capecitabine 0.52 or 2.1 mmol/kg (563 and 2250 mg/m^2, respectively) given once daily for 5 consecutive days/week by oral gavage for 3 weeks

(days 0–4, 7–11, 14–18). Animals were culled on day 0 at 15, 30 min, 1, 2, 4, 8 and 24 h, and prior to planned treatment on days 7 and 14 after the start of treatment. Three animals per time-point were analysed. At the time of collection, blood was collected in heparin, and plasma isolated and stored at −80 °C. The liver was removed immediately and stored in RNAlater® solution (SIGMA, Gillingham, UK). Tumours were macro-dissected to remove fibrotic tissue and blood vessels and snap-frozen in liquid nitrogen.

Tumour response was assessed during the course of treatment by caliper measurements of xenografts taken three times a week in perpendicular diameters from the start of treatment. Tumour volume was calculated as (length * width^2)/2. Results were expressed as mean ± SEM of tumour growth rate (ratio between volume on the day of measurements and tumour volume on the first day of drug treatment) of 16 and 10 xenografts for controls and treated animals, respectively.

2.5. *Determination of capecitabine and metabolite concentrations in plasma, liver and tumour xenografts*

Capecitabine and metabolite concentrations were determined both in plasma and tissues according to our previously published method.[20] Briefly, 50 mg of tissue was homogenised with 250 μl of 50 mM ammonium acetate:acetonitrile (1:3 v/v). After centrifugation at 3500 g for 10 min at 4 °C, the supernatant was transferred into a 200 μl tapered well 96-well microplate and evaporated to dryness. The dried extract was resuspended in 100 μl water and 10 μl was analysed by HPLC. For plasma samples, 50 μl of plasma was mixed with 150 μl of acetonitrile and processed similarly. Compounds were separated on a Develosil ODS-UG-3 column (4.6 × 150 mm, 3 μm) (Nomura Chemical) and mass spectrometry analysis was carried out on a Finnigan TSQ Quantum Discovery mass spectrometer using specific SRM transitions for each compound. Results were expressed as means ± SD values for three samples for each tissue.

2.6. *Statistical analysis*

For the cytotoxicity assays, growth inhibition curves were plotted as a percentage of control cells and IC_{50} estimates were determined by Graphpad Prism 3 Software (Graphpad Software, San Diego, CA) using a sigmoidal curve fitting with variable slope. The goodness of fit determined by r^2 was greater than 0.9 and the Hill coefficient <−1.

The gene expression data analysis of xenograft samples used a one-way ANOVA and Bonferroni multiple comparisons between controls and treated samples. The normality of the distributions was verified prior to the ANOVA.

3. Results

3.1. *Gene expression profiles of capecitabine-metabolising enzymes differ in tumour xenografts, mouse liver and human liver*

In vivo, the initial metabolism of capecitabine takes place in the liver. The level of expression of capecitabine-metabolising enzymes might influence the extent of capecitabine conversion into its active metabolites and therefore which metabolite is delivered to the tumour. A gene expression analysis of capecitabine metabolising enzymes was carried out in mouse liver, HCT 116 colon cancer xenografts and fresh human hepatocytes by qRT-PCR according to a previously published method.[17]

There was a significantly different pattern in gene expression between mouse liver and HCT 116 xenografts (Fig. 1). CES1 and DPD were the most prominent enzymes expressed in mouse liver tissue compared to HCT 116 xenografts (CES1 was not expressed in HCT 116 xenografts). Conversely, the level of CDD gene expression was ~100-fold lower in mouse liver compared to HCT 116 xenografts (GOI/REF = 0.02 and 3.05, respectively). UP expression was similar in mouse liver and HCT 116 xenografts (GOI/REF = 0.45 and 0.62, respectively). TP expression was higher in tumour xenografts (mean GOI/REF = 1.65) than in the mouse liver (mean GOI/REF = 0.6) while DPD expression was ~100 times greater in mouse liver than in HCT 116 xenografts. Overall, the TP/DPD ratios ranged from 0.005 to 0.03 in liver and 5.02–5.36 in HCT 116 xenografts.

To identify potential interspecies differences in drug metabolism, the gene expression was compared between mouse liver and human hepatocytes. The variability between donors was relatively limited with the coefficient of variation ranging from 26% for DPD to 58% for CES1. The major differences in gene expression were observed for CES2 and CDD: CES2 expression was ~100-fold higher in human liver than in mouse liver tissues (GOI/REF = 17 and 0.1, respectively). A similar difference was observed for the expression of CDD (GOI/REF = 2.5 and 0.02, respectively). The consequence of these differences is potentially a greater conversion of capecitabine to 5′DFCR and subsequently to 5′DFUR in human liver, in comparison to mouse liver.

3.2. *CDD, TP and DPD expressions influence both 5′DFCR and 5′DFUR cytotoxicities*

The growth inhibitory effect of capecitabine and metabolites is linked in part to the metabolism of the drug in cancer cells.

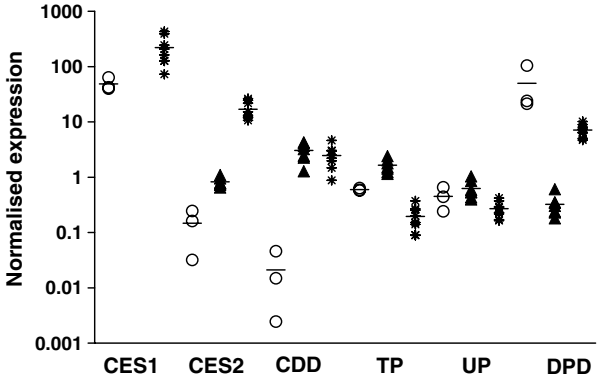

Fig. 1 – Gene expression of capecitabine metabolising enzymes in mouse liver and HCT 116 xenografts. Normalised gene expression was determined by quantitative RT-PCR for CES1, CES2, CDD, TP and DPD in mouse liver (○), HCT 116 xenografts (▲) and human hepatocytes (✳). Note that CES1 was not expressed in HCT 116 xenografts.

Table 1 – Cytotoxicity of capecitabine and metabolites in colon cancer cell lines

	IC_{50} values (μM)							
	CAPE (mean ± SD)		5′-Deoxy-5-fluorocytidine (5′DFCR mean ± SD)		5′-Deoxy-5-fluorouridine (5′DFUR, mean ± SD)		5-Fluorouracil (5-FU, mean ± SD)	
HCT 116	2850	995	57.5	4.80	35.2	2.00	7.70	0.700
HT29	1590	219	186	40.8	57.5	6.10	6.70	0.800
SW620	4190	1150	217	36.0	57.5	6.60	22.3	2.70
HCT8	5970	4200	121	20.0	66.8	11.2	6.70	0.70
HCT15	5840	3970	586	82.0	86.0	12.0	9.90	1.30
COLO205	863	165	59.9	10.0	32.2	7.00	4.90	0.60

The cytotoxic effect of 24-h exposure to capecitabine, 5′DFCR, 5′DFUR and 5-FU was determined by SRB assay. Results are mean ± SD of 3–5 experiments performed in triplicate.

To identify potentially limiting steps in capecitabine metabolism in cells, the cytotoxicity of capecitabine and its metabolites was determined in six colon cancer cell lines using the SRB assay and compared with the gene expression profile of capecitabine-metabolising enzymes in the same cell lines determined by qRT-PCR.

Capecitabine induced a significant cytotoxic effect *in vitro* only at high concentrations (Table 1). Mean IC_{50} values varied from 860 μM in COLO205 cells to 6000 μM in HCT8 cells. Both 5′DFCR and 5′DFUR were significantly more cytotoxic than capecitabine: IC_{50} values ranged from 58 to 590 μM for 5′DFCR and 32 to 86 μM for 5′DFUR. The distribution of IC_{50} estimates for 5′DFCR was greater across the six cell lines (10-fold range) than for 5′DFUR (<3-fold range). Finally, 5-FU IC_{50}s varied 4.5-fold between the most sensitive (COLO205) and least sensitive (SW620) cell lines.

Gene expression analysis of capecitabine-metabolising enzymes, i.e. CES1 and 2, CDD, TP, DPD was also carried out using qRT-PCR (Table 2). CES1 was significantly expressed only in HCT8 and COLO205 cells (normalised CES1 = 1.29 and 1.24, respectively). CDD expression varied 10-fold across the cell lines, COLO205 expressing the highest levels (normalised CDD = 2.54) and HCT-15 the lowest (normalised CDD = 0.22). TP was expressed significantly only in HCT 116 and HT29 cells (normalised TP = 0.38 and 0.33, respectively). Finally, both HT29 and HCT-15 cells express high levels of DPD (normalised DPD = 0.93 and 1.19, respectively). Overall, only HCT 116 and HT29 cell lines expressed significant levels of all the enzymes involved in the metabolic pathway of capecitabine.

Table 2 – Gene expression analysis of capecitabine metabolising enzymes in colon cancer cell lines

	CES1	CES2	CDD	TP	DPD
HCT 116	<LOQ	0.30	1.05	0.38	0.13
HT29	<LOQ	0.53	0.25	0.33	0.93
SW620	0.02	1.27	0.28	<LOQ	<LOQ
HCT8	1.29	0.89	1.78	<LOQ	0.01
HCT15	<LOQ	0.61	0.22	<LOQ	1.19
COLO205	1.24	0.31	2.54	0.05	<LOQ

CES1, CES2, CDD, TP and DPD mRNA expressions were determined by qRT-PCR as previously published [Macpherson, 2006 #178]. Gene expressions are normalised by the geometric mean of 18S, B2M, GAPDH and POLR2 reference genes. Results are mean ± SD of 2 independent experiments performed in triplicate. <LOQ: values lower than limit of quantification.

When the ratios of 5′DFCR and 5′DFUR IC_{50} estimates were plotted against the mRNA expression of CDD, cell lines which displayed low expression (<1.5) had a high ratio (>2) suggesting that conversion of 5′DFCR within the tumour cells was a major factor determining 5′DFCR cytotoxic effect (Fig. 2A). Moreover, the difference in 5′DFUR IC_{50} estimates between cell lines was unrelated to TP expression. However, the ratio of TP and DPD expressions did correlate with 5′DFUR IC_{50} (Fig. 2B): Four cell lines expressing TP/DPD ratios lower than 0.6 displayed significantly higher 5′DFUR IC_{50} values (>50 μM) than the two cell lines presenting ratios greater than 3. However, the IC_{50} values for these two cell lines were very similar (~33 μM) despite a ~6-fold difference in the ratios. No correlation was observed between UP or UP/DPD and 5′DFUR cytotoxicity.

3.3. *Difference in gene expression is consistent with pharmacokinetic profiles in plasma, liver and tumour xenografts*

To establish whether the differences in gene expression would impact on metabolism *in vivo*, a pharmacokinetic/pharmacodynamic study was carried out in mice bearing HCT 116 xenografts receiving 0.52 and 2.1 mmol/kg/d of capecitabine by oral gavage. Capecitabine administered at 0.52 mmol/kg/day induced partial control of HCT 116 xenografts tumour growth: growth rate = 7.5 ± 0.5 on day 21 (Fig. 3A). Capecitabine 2.1 mmol/kg/day achieved complete control of tumour growth during the treatment period: growth rate = 1 ± 0.2 on day 21 (this dose of capecitabine is similar to the dose used in patients).

The pharmacokinetic study showed that 5′DFCR was the most abundant metabolite in both plasma (Fig. 3B) and liver (Fig. 3C): C_{max} = 110 ± 10 μM in plasma and 590 ± 140 μM in liver. When considering the concentrations 0–8 h after administration, there was a significant correlation between liver and plasma concentrations of 5′DFCR at both dose levels (r^2 = 0.91, n = 18 and r^2 = 0.95, n = 18 at 0.52 and 2.1 mmol/kg/day of capecitabine, respectively). The low CDD expression observed in mouse liver could therefore be a limiting factor to the conversion of 5′DFCR in the liver.

The residual concentrations determined in liver and tumour tissues on day 7 (Fig. 3D), 72 h after the previous oral

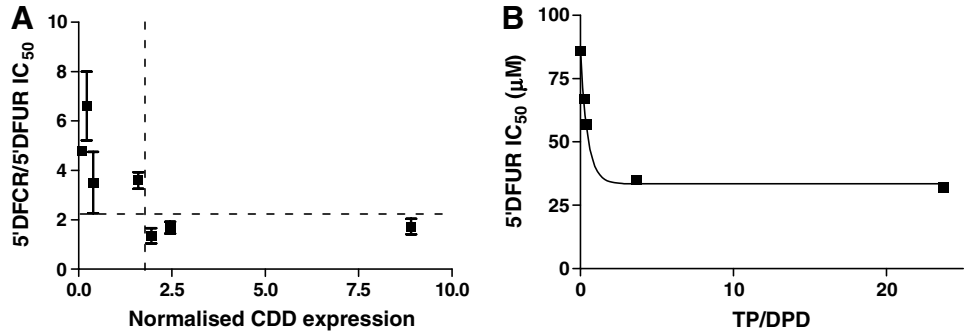

Fig. 2 – Impact of CDD and TP/DPD on capecitabine metabolites cytotoxicity. (A) The ratio of IC_{50} values for 5′DFCR and 5′DFUR is plotted against CDD mRNA expression in six colon cancer cell lines. (B) Correlation between TP/DPD ratios and cytotoxicity of 5′DFUR.

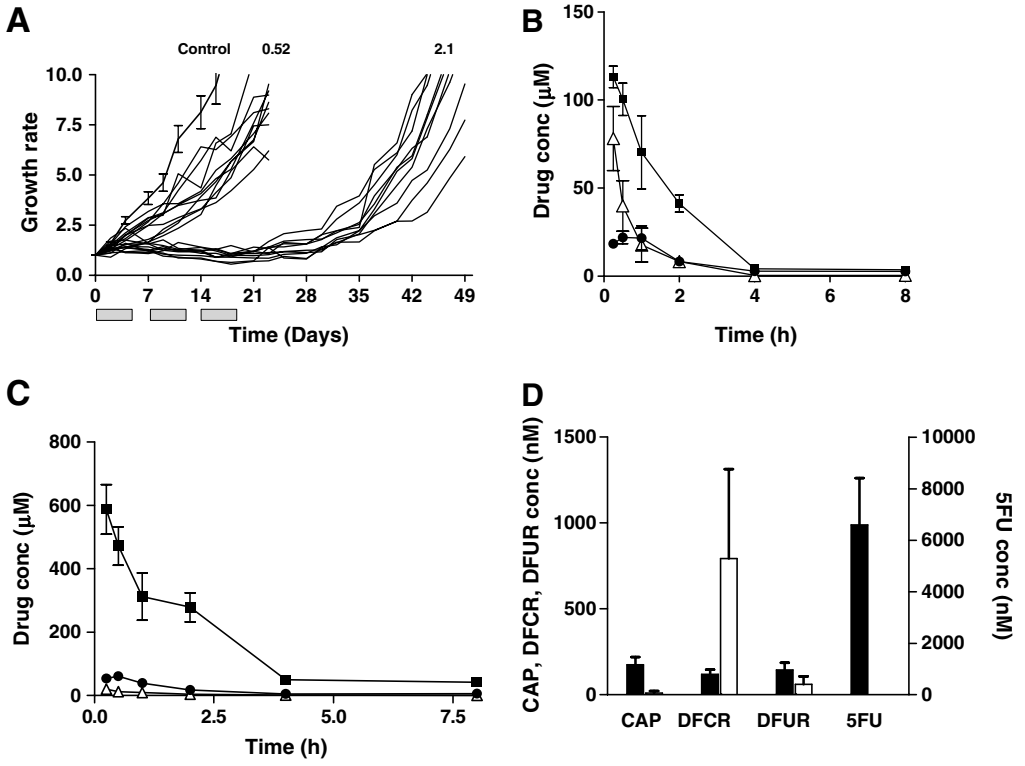

Fig. 3 – Pharmacokinetic/pharmacodynamic study with capecitabine. (A) capecitabine efficacy in HCT 116 xenografts. HCT 116 cells were implanted subcutaneously in nude mice. Animals received either capecitabine 0.52 or 2.1 mmol/kg once daily by oral gavage for consecutive 5 days/week for 3 weeks (days 0–4, 7–11, 14–18). The growth rate is presented for each individual xenograft. (B) Capecitabine and metabolite concentrations in plasma. (C) Liver tissue after the first dose of capecitabine (2.1 mmol/kg); capecitabine (△), 5′DFCR (■), 5′DFUR (●). (D) Concentrations of capecitabine (CAP), 5′DFCR, 5′DFUR and 5-FU in liver tissue (white bars) and HCT 116 xenografts (black bars) on day 7 of treatment.

dose of capecitabine (2.1 mmol/kg/day) administered on day 4, were unexpected, demonstrating high concentrations of 5′DFCR in the liver (790 nM). 5′DFCR was also demonstrable in the tumour, albeit at a lower concentration (120 nM) than in the liver. Also, surprisingly high concentrations of 5-FU were present in the tumour (mean 6600 nM) at this time-point, whilst 5-FU was undetectable in either the liver or the plasma. 5-FU was also detectable in the tumours of animals treated with capecitabine 0.52 mmol/kg/day: 2200, 4000 and 4800 nM (mean values on days 7, 14 and 21, respectively (data not shown)).

A gene expression analysis was carried out in tumour xenografts during the course of the treatment. TP/DPD increased over time in xenografts exposed to 2.1 mmol/kg/day of capecitabine: From 6.6 on day 7 to 10 on day 21 while xenografts exposed to 0.52 mmol/kg/day of capecitabine increased slightly from 3.1 on day 7 to 5.3 on day 21. There was a significant correlation between tumour 5-FU concentrations (days

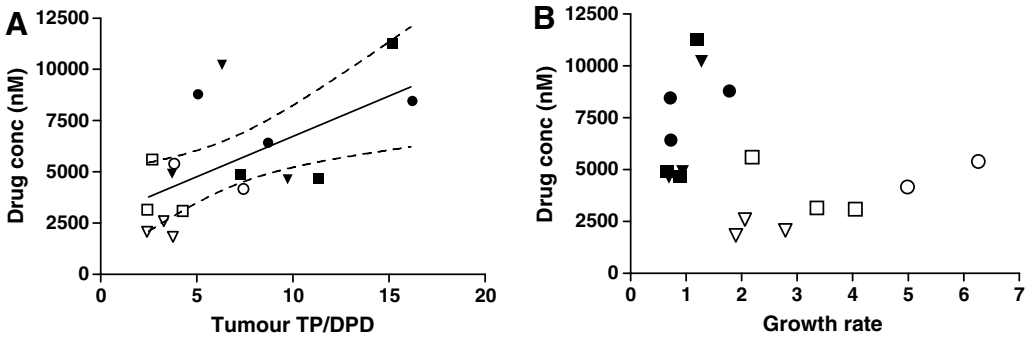

Fig. 4 – Pharmacokinetic/pharmacodynamic relationships after administration of 0.52 mmol/kg/d (open symbols) and 2.1 mmol/kg/d (close symbols) of capecitabine. Samples were collected on day 7 (▽, ▼), day 14 (□, ■) and day 21 (○, ●) after administration. (A) Correlation between 5-FU concentrations in xenograft tumours and TP/DPD ratios. The correlation line and 95% confidence intervals are presented. (B) 5-FU concentrations in xenograft tumours and tumour response. Individual xenografts data are presented.

7, 14 and 21 at both dose levels) and TP/DPD gene expression ($r^2 = 0.37$, $p = 0.01$, $n = 16$) (Fig. 4A). No other correlations were observed with 5′DFCR, 5′DFUR or capecitabine. In addition, no correlation was observed between TP or UP expression and either capecitabine or metabolite concentrations.

Moreover, 5-FU concentrations were significantly related to tumour response (Fig. 4B): Xenografts exposed to 2.1 mmol/kg/d of capecitabine leading to 5-FU concentrations >4000 nM showed constant control of tumour growth (growth rate <2 at all time). Conversely, xenografts exposed to 0.52 mmol/kg/d of capecitabine with lower tumour 5-FU concentrations showed a progressive increase in growth rate over time. Even when 5-FU concentrations reached 4000 nM on day 21, the growth rate was high. This suggests that early control of tumour growth with high concentrations of 5-FU in the tumour is necessary for growth control.

4. Discussion

Capecitabine undergoes extensive metabolism in both liver and tumour. The studies presented here were undertaken to test the hypothesis that gene expression analysis between different target tissues could help to predict interspecies differences, potential variability between patients and tumour response.

The gene expression data presented in this study are consistent with enzymatic activities previously reported by Tsukamoto et al., in particular the low expression of CDD in mouse liver and its greater enzymatic activity in human compared to mouse liver.[21] Similar correlations were previously shown for DPD, TS and TP, allowing the use of quantitative RT-PCR (qRT-PCR) as an indirect measure of enzyme activities.[22]

In *in vitro* studies determining the growth inhibition of capecitabine and metabolites in six colon cancer cell lines, CDD expression correlated with 5′DFCR cytotoxicity, consistent with previously published data.[11] Cell lines demonstrating high TP/DPD ratios also showed greater sensitivity to 5′DFUR.

Differential gene expression between mouse liver and HCT 116 xenografts was able to explain the different pharmacokinetic profiles for capecitabine and metabolites in plasma, liver and xenografts: high carboxylesterase expression in the liver facilitated the efficient conversion of capecitabine to 5′DFCR, consistent with low capecitabine concentrations in the liver compared to 5′DFCR concentrations. However, the very low level of hepatic CDD in the mouse liver became a limiting factor in 5′DFCR conversion, resulting in higher concentrations of 5′DFCR than 5′DFUR in the plasma. High DPD expression in mouse liver in conjunction with relatively low TP expression explained the absence of 5-FU in the liver, as any 5-FU which was formed would have been rapidly converted to dihydro-5-FU and other metabolites. In HCT 116 xenografts, the absence of CES led to significant concentrations of capecitabine in the tumour tissue. Concentrations of 5′DFUR were higher than 5′DFCR due to high CDD expression. As previously observed by Ishikawa et al., 5-FU was the predominant metabolite in tumour.[12] In contrast to the situation in liver, high TP expression associated with low DPD expression favoured 5-FU formation over its detoxification in this particular xenograft model.

The low efficiency of 5′DFUR conversion in mouse liver by both UP and TP observed in this study was first identified by El-Kouni et al.[23] The same study showed that human hepatic TP and not UP was the main enzyme converting 5′DFUR in liver. More recently, UP knock-out mice, which present high plasma and tissue uridine concentrations, were shown to tolerate 5-FU (85 mg/kg) better than wild type animals, suggesting that UP plays an important role in the anabolism of 5-FU.[24] However, neither 5′DFUR nor capecitabine was tested in that study. In tumours and cell lines, a large body of literature focuses on TP as a determinant for tumour-specific formation of 5-FU after capecitabine administration.[12,13]However, uridine phosphorylase can convert 5′DFUR into 5-FU in human and mouse tumour cell lines.[25] In this study, TP expression was ~3-fold higher than UP in HCT 116 xenografts but no correlation was observed between TP or UP expression and 5′DFUR cytotoxicity. However, *in vitro*, cell lines presenting high TP/DPD ratios showed a greater sensitivity to 5′DFUR. Moreover, *in vivo*, 5-FU concentrations in the tumour also correlated with TP/DPD expression. This is consistent with results from previous pre-clinical studies using human cancer

xenografts and correlating TP/DPD ratios with the efficacy of capecitabine and 5′DFUR.[26]

The gene expression analysis identifies CDD as an important factor for the delivery of 5′DFUR to the tumour. The greater CDD expression observed in fresh human hepatocytes as compared to mouse liver predicts the greater conversion of 5′DFCR into 5′DFUR. Higher concentrations of 5′DFUR were indeed reported by Mader et al. in human plasma.[27] This confirmed the potential role of CDD in clinical outcome following capecitabine administration. CDD polymorphisms induce different metabolic activity and their potential importance in relation to capecitabine therapy merits further study.[28]

These studies have confirmed the importance of the expression of drug metabolising enzymes in the pharmacokinetics and activity of this commonly used agent. TP/DPD is a predictive marker of sensitivity and its variations over time, although important, are related to tumour 5-FU concentrations. Whilst the invasive assessment of gene expression and fluoropyrimidine PK in normal liver and metastatic cancer tissue using sequential biopsies in patients is not feasible, advances in non-invasive imaging using 19F imaging using magnetic resonance spectroscopy might allow a more detailed monitoring of capecitabine PK in individual patients. A recent study by Klomp et al.[29] has demonstrated the feasibility of such an approach.

Conflict of interest statement

Prof. Jodrell is a member of the editorial board of the Xeloda website (Roche Pharmaceuticals) and member of the Data Monitoring and Safety Board for CHAT study (Honoraria paid). Prof. Jodrell has received departmental support for clinical trials with capecitabine (Roche Pharmaceuticals).

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