Antiangiogenic activity of the selective cyclooxygenase 2 inhibitor rofecoxib in human colorectal cancer liver metastases

We have previously reported that colorectal cancer (CRC) liver metastases from patients, who had received the selective cyclooxygenase 2 (COX-2) inhibitor rofecoxib (Vioxx) 25 mg daily for a mean period of 26 days, had a 31% lower mean microvessel density compared with tumours from individuals who had received placebo only.¹ These data suggest that one mechanism of action of rofecoxib in human CRC liver metastases might be inhibition of angiogenesis. Therefore, we investigated the potential antiangiogenic activity of rofecoxib in human CRC liver metastases by dynamic contrast enhanced magnetic resonance imaging (DCE-MRI), before and after treatment with oral rofecoxib 25 mg daily for 28 days. As short term treatment of rats with the selective COX-2 inhibitor celecoxib is associated with an increase in serum levels of the endogenous antiangiogenic factor endostatin,² we also measured serum levels of endostatin and the proangiogenic factor vascular endothelial growth factor (VEGF), at the same time as DCE-MRI.

Patients undergoing liver resection of CRC liver metastases were enrolled if diagnostic MRI identified a single metastasis greater than 1.5 cm in diameter that was not adjacent to a major vessel. Other inclusion/exclusion criteria were as described previously.¹ A detailed description of the DCE-MRI technique using gadolinium-diethylenetriaminepenta-acetic acid (Gd-DTPA) is available from the corresponding author. Tumour vascularity was measured according to Cancer Research UK Pharmacodynamic/Pharmacokinetic Technologies Advisory Committee recommendations.³ The initial area under the Gd-DTPA concentration-time curve at 60 seconds (IAUGC in mmol/lxs) was determined and the concentration-time curve was fitted to the Tofts model⁴ in order to calculate the transfer constant K_{trans} (the permeability-surface area product/second), using an aortic arterial input function.

Five patients had completed the study when it was terminated prematurely on 30 September 2004 following the worldwide withdrawal of rofecoxib. One DCE-MRI examination was affected by incomplete administration of Gd-DTPA. Therefore, DCE-MRI data from this patient were not analysed further. In all four patients, the aortic IAUGC at 60 seconds measured after rofecoxib therapy was higher than the corresponding pretreatment value (mean difference in IAUGC 26.1 (95% confidence interval (CI) -4.0 to +56.1) mmol/lxs; $p=0.07$, paired t test). One possible explanation is the ability of rofecoxib and other selective COX-2 inhibitors to promote fluid retention and increase systemic arterial blood pressure. Future MRI studies of the anticancer activity of coxibs will also need to address potential effects on systemic arterial haemodynamics.

Unlike previous DCE-MRI studies of human CRC liver metastases,⁵⁻⁸ we calculated IAUGC and K_{trans} values for anatomically defined peripheral and central regions of interest (ROI) for each tumour. IAUGC and K_{trans} values in the peripheral region of untreated metastases were invariably higher than the corresponding central area of each tumour (table 1), consistent with reduced blood flow at the centre of the metastases. Treatment with rofecoxib 25 mg daily for 28 days resulted in a decrease in peripheral IAUGC (mean 15.6% decrease (95% CI -44.6 to +13.5%); $p=0.16$) and K_{trans} (mean 36.4% decrease (95% CI -59.4 to -13.4%); $p<0.01$) in all four cases (table 1), consistent with DCE-MRI studies of CRC liver metastases using other putative antiangiogenic agents.⁹ The effect of rofecoxib on central IAUGC and K_{trans} values was more variable. There was an increase in central ROI IAUGC and K_{trans} in two tumours following rofecoxib treatment (table 1).

A decrease in peripheral tumour K_{trans} is consistent with antiangiogenic activity of rofecoxib in vivo, secondary to decreased tumour blood flow and/or a reduction in vessel permeability. Recently, the concept of vascular "normalisation" has been put forward.⁹ A decrease in vascular "leakiness" in peripheral tumour tissue and/or increased blood flow in central hypoxic areas of metastases are both compatible with this emerging

concept of antiangiogenic therapy. It will be important to obtain further evidence supporting the concept of vascular "normalisation", by tumour interstitial fluid pressure measurements and analysis of the pericyte coverage index, in future translational studies of other selective COX-2 inhibitors.

Following rofecoxib treatment, there was no significant difference in serum VEGF levels (fig 1A). However, serum endostatin level increased (mean 25.3% (95% CI 10.2-40.5%); $p=0.02$) in all cases (fig 1B). A relatively small (1.6-fold) increase in circulating endostatin levels has been reported to result in significant inhibition of angiogenesis and tumour growth in transgenic mice overexpressing endostatin.¹⁰ Therefore, one working hypothesis is that antiangiogenic activity of rofecoxib is mediated by increased levels of endogenous antiangiogenic factors such as endostatin. Our data on a small number of patients with CRC liver metastases (limited by withdrawal of rofecoxib from clinical use) should prompt a thorough investigation of the magnitude and mechanistic basis of the effect of coxibs on systemic and tissue levels of endostatin in cancer patients.

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Table 1 Effect of rofecoxib on dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) of human colorectal cancer (CRC) liver metastases

		IAUGC (mmol/lxs)			K_{trans} (/s)		
		Pre-rofecoxib	Post-rofecoxib	Δ IAUGC‡	Pre-rofecoxib	Post-rofecoxib	Δ K_{trans} †
Peripheral§	1	23.0*	22.0	-1.0 (-4.3)	0.0180**	0.0120	-0.0060 (-33.3)††
	2	27.0	26.4	-0.6 (-2.2)	0.0124	0.0088	-0.0036 (-29.0)
	3	28.0	24.1	-3.9 (-13.9)	0.0128	0.0095	-0.0033 (-25.8)
	4	21.3	12.4	-8.9 (-41.8)	0.0059	0.0025	-0.0034 (-57.6)
Central§	1	6.3	10.8	+4.5 (+71.4)	0.0040	0.0050	+0.0010 (+25.0)
	2	12.9	12.6	-0.3 (-2.3)	0.0051	0.0035	-0.0016 (-31.4)
	3	14.6	19.8	+5.2 (+35.6)	0.0057	0.0092	+0.0035 (+61.4)
	4	9.2	5.7	-3.5 (-38.0)	0.0019	0.0010	-0.0009 (-47.4)

*The last dose of rofecoxib was taken approximately four hours before the post-treatment DCE-MRI.

‡Absolute difference between pre- and post-rofecoxib values. Values in parentheses represent the percentage change from baseline (pre-rofecoxib). Metastases (mean (SEM) pretreatment maximum tumour diameter 4.0 (1.2) cm) did not change in size significantly during the four week study period (mean (95% confidence interval) difference in maximum tumour diameter following rofecoxib treatment -0.2 (-0.5 to 0.1) cm; $p=0.16$). Therefore, the same regions of interest (ROI) were used for pre- and post-rofecoxib DCE-MRI.

§Data are presented separately for peripheral and central ROI of each numbered (1-4) metastasis. All DCE-MRI data were analysed by a single investigator, blinded to the identity of the data sets. ROI were identified for the peripheral (outer 25%) and central (inner 75%) areas of each metastasis, which were used for both pre- and post-treatment analyses.

* $p<0.01$ and ** $p=0.03$ for comparison of peripheral versus central initial area under the Gd-DTPA concentration-time curve (IAUGC) and K_{trans} (the permeability-surface area product/second) values, respectively, in pretreatment metastases.

†† $p<0.01$ for the difference between pre- and post-treatment peripheral K_{trans} values.

Figure 1 Effect of rofecoxib on serum levels of vascular endothelial growth factor (VEGF) (A) and endostatin (B), measured by enzyme linked immunoassay (R&D Systems, Abingdon, UK). Lines join paired data from individual patients before (pre) and after (post) rofecoxib therapy (n = 5). Symbols for each set of paired data correspond to the same metastases in (A) and (B).

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Ulcerative colitis during CTLA-4Ig therapy in a patient with rheumatoid arthritis

The coexistence of rheumatoid arthritis (RA) and ulcerative colitis (UC) is exceptional, with only a few cases reported in the literature.¹⁻⁴ However, the current use of biological agents can modify the balance of inflammatory mediators and the lymphocytic profiles of the original disease, promoting the emergence of additional conditions. This seems to be the case for a patient with severe RA who developed UC while being treated with CTLA-4Ig (abatacept).

We report the case of a 55 year old male with a seven year history of seropositive RA refractory to treatment with 20 mg/week methotrexate (MTX), 1.5 g/day sulphasalazine (SSZ), 400 mg/day hydroxychloroquine, and 100 mg/day azathioprine. The patient was included in an CTLA-4Ig (10 mg/kg intravenously monthly) plus MTX clinical trial. All other disease modifying antirheumatic drugs were discontinued. During treatment, major clinical and serological responses were achieved (table 1). Fifteen months later, the patient developed diarrhoea, rectal bleeding, crampy abdominal pain, tenesmus, and weight loss. CTLA-4Ig and MTX were discontinued. Stool search for bacteria, ova, and parasites yielded negative results; serological tests for human immunodeficiency virus, hepatitis B, and hepatitis C were non-reactive. Colonoscopy revealed extensive ulcerative lesions in the sigmoid colon and rectum. Histology showed a dense lymphoplasmocytic inflammatory infiltration distorting the architecture of the mucosa; cryptitis with irregular basal branching and intraluminal abscesses were also apparent and Paneth cells were

identified in crypt bases. A diagnosis of UC was made and oral mesalamine therapy was started at a dose of 1.6 g/day with a satisfactory response. The dose of mesalamine was gradually reduced over the next three months until discontinuation.

Four months after CTLA4-Ig withdrawal, the patient remained asymptomatic for UC and the search for blood in stool was negative. However, arthritis was reactivated and a step on regimen with 25 mg/week MTX, 3 g/day SSZ, 5 mg/day prednisone, and 20 mg/day leflunomide was initiated.

Diverse methods have been proposed to assess the likelihood of a causal connection between environmental exposure (including therapeutic drugs) and an undesirable adverse event.⁵ According to Miller and colleagues,⁶ a series of five primary attribution elements or pertinent questions should be addressed when suspected exposure may be related to a clinical outcome for a given patient: (1) temporal association; (2) lack of likely alternative explanations; (3) dechallenge (improvement in symptoms following discontinuation of the drug); (4) rechallenge; and (5) biological plausibility.

In our case, both temporal association and dechallenge suggest that CTLA-4Ig could be related to the development of UC and autoantibody induction. Analysing other likely alternative explanations, the major infectious causes of bloody diarrhoea were excluded, and no evidence of vasculitis of the mesenteric arteries or amyloidosis was found. Rechallenge was not available because CTLA-4Ig was not reinitiated.

Biological plausibility is also possible considering the following facts: CTLA-4Ig was designed and has been used to block the interactions between CD80/CD86 on antigen presenting cells and CD28 on T lymphocytes, preventing their activation.⁷ However, this same mechanism could alter both the development and maintenance of regulatory T cells,⁸ which control intestinal inflammation. Also, absence of the cytoplasmic domain of CTLA-4 and hence of the intracellular signal it mediates favours a Th2 biased phenotype.⁹ This could explain the emergence of UC and the presence of autoantibodies, both phenomena related to a Th2 response.¹⁰

Although four of Miller's primary attribution elements are fulfilled, we cannot prove causality in this single case, especially in the absence of rechallenge. Nevertheless, theoretically, CTLA-4Ig could favour the emergence of Th2 mediated phenomena, an issue that needs to be elucidated.

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Table 1 Clinical and laboratory findings

Measurement	CTLA-4Ig therapy				
	Baseline	12 months	15 months	19 months	26 months
Tender joint count	22/28	9/28	11/28	16/28	28/28
Swollen joint count	18/28	2/28	8/28	12/28	24/28
C reactive protein (mg/l)	22.7	7.3	5.6	42.7	50.2
DAS-28	6.58	4.25	4.77	6.16	7.47
Antinuclear antibodies*	0	1:40	1:40	0	0
Anti-dsDNA (UI/ml)†	42	13	107	45	30

*Antinuclear antibodies are expressed in dilutions. Indirect immunofluorescence on HEp-2 slides.

†Anti-dsDNA, anti-double stranded DNA antibodies (reference range ≤ 99 UI/ml).