Susceptibility MRI of Vascular Function and Therapeutic Response In LS174T Colorectal Carcinomas Grown Orthotopically in Murine Liver.

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Introduction:

The majority of tumour models used in cancer research are used as investigative tools for primary cancers, whilst relatively little research focuses on metastatic disease. Furthermore, few studies have assessed metastases regarding their respective host microenvironment, their degree of host vessel co-option and vascular architecture. Therefore little is known about the microcirculation and therapeutic responses within these tumour models. This study aims to investigate LS174T colorectal carcinomas grown as orthotopically implanted liver metastases within murine liver using susceptibility MRI, which has previously been used to investigate tumour vascular architecture and function of subcutaneous tumour models[1,2]. The effective MRI transverse relaxation rate R_2* was measured using i) intrinsic-susceptibility MRI, sensitive to changes in endogenous [deoxyhaemoglobin] induced by either carbogen (95%O_2/5%CO_2) breathing (Experiment 1), or 24 hours post administration of the vascular disrupting agent ZD6126 (Experiment 2), and ii) susceptibility-contrast enhanced MRI using the superparamagnetic iron oxide (SPIO) contrast agent Endorem. As the SPIO particles (>150 nm) are smaller in size than that of the intrinsic contrast agent (red blood cells, 6 µm) they are more likely to be able to traverse tortuous tumour capillaries, therefore changes in tumour R_2* induced by Endorem may provide an assessment of vascular volume[1].

Methods:

Adult female MF1 nude mice were inoculated with $1x10^7$/0.1mL LS174T cells intrasplenically. Resulting liver metastasis were imaged ca. 25 days later (tumour volume ca. 0.8cm^3). Mice were anaesthetized using an i.p injection of Hypnorm/Hypnovel and a tail vein cannulated for administration of 2.5mgFe/kg Endorem. MRI was performed on a 4.7T Varian Unity Inova system using a quadrature birdcage coil. Air or carbogen was administered at 2l/min via a nosepiece. Multi gradient-echo (MGRE) images were acquired from three 1mm thick contiguous slices through the liver with TR = 80 ms, initial TE = 4 ms, TESPACE = 3 ms, No.of echoes= 8, AQ = 8 mins. For Experiment 1, MGRE images were acquired whilst the host initially breathed air, then carbogen, resumed air breathing and finally following administration of Endorem. For Experiment 2, MGRE images were acquired pre- and post-injection of Endorem, 24 hours prior to and after administration of either 200mg/kg ZD6126 or saline i.p. R_2* maps were generated by fitting a single exponential to the signal intensity voxel-by-voxel. The mean R_2* from each individual tumour within each slice during each challenge was determined from a region of interest (ROI) encompassing the whole tumour but excluding surrounding normal tissue. ROI's were also derived from the remaining normal liver parenchyma to establish the carbogen response within the host tissue. Following MRI, mice were administered 15mg/kg of the perfusion marker Hoechst 33342 i.v and allowed to circulate for 1 minute before sacrifice and liver harvesting for subsequent fluorescence and H & E light microscopy.

Results and Discussion:

Experiment 1: A heterogeneous tumour response to both carbogen and Endorem was typically observed, with Endorem inducing a dramatic loss of signal in the remaining normal liver (Figure 1). The data are summarised in Table 1 (mean ± 1 s.e.m.). Carbogen breathing induced a significant (**p<0.01, Student's paired t-test) increase in R_2* in all metastases, consistent with an increase in [deoxyhaemoglobin]. One explanation for this is that carbogen induces a transient increase of blood flow in the remaining normal, well-vascularized liver tissue as a result of CO_2-induced vasodilation, causing a redistribution of blood flow away from the tumour ("steal effect")[1,3]. This hypothesis is supported by the observed decrease in R_2* in normal liver parenchyma during carbogen breathing indicated by the arrow in Figure 1, and consistent with previous observations[4]. Administration of Endorem resulted in a significant increase in R_2* indicating the presence of a significant tumour blood volume.

Experiment 2: The baseline R_2* maps indicate an increase in the size of the tumour over 48 hours, a reduction in tumour R_2* after treatment, and also a slight increase in R_2* after Endorem injection (Figure 2). The data are summarised in Table 2 (mean ± 1 s.e.m.). Tumour R_2* showed a small yet non-significant decrease after 24 hours irrespective of treatment. In the untreated tumours this may be indicative of a decrease in tumour [deoxyhaemoglobin] related to an increase in vascularity over the 48 hour period. Hoechst 33342 images showed the presence of relatively few, large perfused blood vessels within the control metastases, which when stained by H & E co-localized with areas of viable tumour tissue. These vessel structures were absent within the core of ZD6126 treated tumours, and these tumours presented large areas of central necrosis with a viable rim at the tumour periphery consistent with previous observations[5,6]. The slight decrease in R_2* is therefore most likely due to central necrosis. The inoculation of Endorem revealed similar blood volumes to that seen in Experiment 1, which showed no apparent reduction after treatment with ZD6126. However, the blood volumes within the orthotopic LS174T liver metastasis are relatively small compared to more densely vascularized tumours as shown by previous studies with well perfused subcutaneous rat xenographs[7]. ZD6126 dramatically reduced R_2* and caused massive central haemorrhagic necrosis within these tumours which further reflects the higher degree of baseline necrosis present within the orthotopic metastases. Therefore changes in blood volume caused by the administration of ZD6126 may not be great enough to reduce the R_2* of the whole tumour within this model.

Figure 1

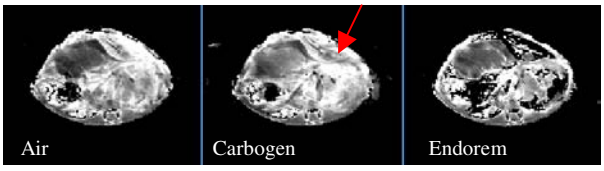

Table 1

(n=9)	Air	Carbogen	Endorem
Mean R_2* (s^{-1})	58.4 ± 4.5	65.2 ± 5.2 **	65.6 ± 5.4 **
ΔR_2* (s^{-1}) to Air	-	6.7 ± 1.5	7.1 ± 3.1

Figure 2

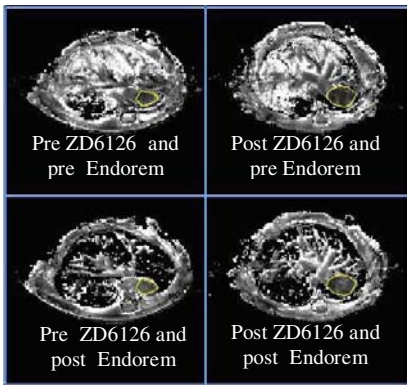

Table 2

		Pre Treatment & Pre Endorem	Pre Treatment & Post Endorem	Post Treatment & Pre Endorem	Post Treatment & Post Endorem
Saline (n=6)	**Mean R_2* (s^{-1})**	73.9 ± 7	78.1 ± 8	65.7 ± 4	70.6 ± 5
	ΔR_2* (s^{-1})	-	4.5 ± 3	-8.2 ± 5.7	5.2 ± 2
ZD6126 (n=10)	**Mean R_2* (s^{-1})**	76.6 ± 5	80.2 ± 4	68.4 ± 3	74 ± 4.5
	ΔR_2* (s^{-1})	-	6.9 ± 2	-8.2 ± 5.2	6.7 ± 3

Conclusions:

The data suggests that changes in R_2* induced by susceptibility MRI methodologies can be used to assess the functionality and therapeutic responses of orthotopic tumour models. The results are complex as the metastatic tumours exhibit heterogeneous biological and metastastic properties, therefore the outcome of how a tumour behaves to intrinsic or therapeutic challenges is dependent on both the tumour properties and the host microenvironment. As the induction of metastases in different organs will present differently, a range of orthotopic models may therefore be needed to assess this approach further.

References

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