Structural spectroscopic analysis of tumour vasculature before and after contrast agent administration to mice

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Background

- Cancer was the second cause of death in the US in 2006. [1]

![Table of the number and cause of deaths in the US in 2006.](image)

- There are more than 293,000 new cases of cancer (excluding non-melanoma skin cancer) diagnosed each year in the UK. [2]
Background

• The conventional cancer treatments, which include many modalities, have a specific goal: to directly kill tumour cells or prevent their proliferation. Tumour cells proliferation and sensitivity characteristics are assessed by pharmacodynamic studies.

• However, the tumour population is unstable and its behaviour is often affected by its own genetic, epigenetic and micro-environmental heterogeneity. Due to this fact, it is thought that current therapeutic attempts to target the expanding array of tumour expressions with customized molecular attacks might be relying in parameters which are not immutable over time.

• As an alternative, therapy directed against tumour vasculature does not exploit tumour cell sensitivities, but relies on the tumour vasculature inhibition which will hopefully lead to tumour suppression.

• Becomes a question of not aiming to the heterogeneous, unconstrained tumour population directly, but using the relatively homogeneous and constrained endothelial population by antiangiogenic therapy, allowing the disregard a vast array of spatial and temporal details of tumour cell expression.[3]
Background

• For more than 60 years it has been suggested that solid tumours require a functioning network of blood vessels in order to sustain growth through provision of oxygen and nutrients and also to remove toxic products of cellular metabolism.

• Angiogenesis (the formation of new blood vessels from the endothelium of existing vasculature) became a target after observations by Folkman in the early 1970s.

• It is now recognised for fact that 1-2mm³ tumours to keep growing need a so-called angiogenic switch which leads to the formation of new blood vessels (neovascularisation).

• Based on this, research unveiled key molecules in this process, such as vascular endothelial growth factor (VEGF) and its receptors. [4]
Background

• To prevent the growth of new vessels in solid tumours, anti-angiogenic drugs have been developed and some sent to clinical trials.

• In cases where the tumour has already a considerable size, a different approach is taken. In this case, by causing rapid vascular shutdown to the existing tumour vasculature it is expected that this cause secondary tumour cell death.

• One of the low molecular weight drugs that show to induce vascular damage and haemorrhagic necrosis in tumours is a tubulin binding agent called Disodium combretastatin A-4 3-O-phosphate (CA-4-P). [5]

• Using Dynamic Contrast Enhance (DCE)-MRI techniques, it is possible to evaluate tumour blood vessels, namely the microvascular response of tumours to the physical and/or chemical treatments, both before and after treatment. [6]
Background

- The literature often refers about CA-4-P an effect of tumour necrosis which is more evident in tumour centre. Even at the most effective doses, the periphery of the tumour keeps a small rim of viable cells, from which tumour regrowth occurs. [5]

- However, this difference of effect between the centre and periphery of the tumour is not well established yet.
Background

- I.e. in [5] the blood flow response of peripheral and central tumour regions, extracted from autoradiographic analysis of tumour sections, showed a very similar reduction in blood flow to central and peripheral tumour regions one hour after treatment with 10mg/kg of CA-4-P. Although there was a tendency for a faster and more efficient recovery of the blood flow during time in the periphery than in the central area of the tumour. [5]

*Figure 2:* The effect of 10mg/kg CA-4-P on blood flow to central (a) and peripheral (b) regions of the tumour, as determined by autoradiographic analysis of tumour sections. Values are the mean± 1SE. The shaded bar represents the limits of SE for the control animals. A significant difference from control (p<0.05) is represented by (*). Solid symbols represent blood flow to untreated tumours from rats sham-treated on day 0.[5]
Background

• I.e. in [6] the effect of CA-4-P treatment on the ratio (central AUC):(peripheral AUC) was studied using 30mg/kg of CA-4-P and DCE-MRI. Central AUC was calculated from pixels more than 5.0 mm from the tumour edge while peripheral AUC was calculated from pixels less than 2.0 mm from the edge. The initial ratio is approximately 1, but decreases significantly at 1 and 6 hours after CA-4-P injection (P≤0.05, paired t test) due to a larger vascular effect in the tumour centre compared with that in the periphery. Nevertheless, significant decreases in AUC were observed in the tumour periphery (two- to four-fold), which compared with 10- to 20-fold AUC decreases in the centre. No significant changes were seen in central tumour AUC, peripheral AUC, or their ratio for saline-treated animals (Fig 3).[6]

Figure 3: Effect of saline (○) or combretastatin A4 phosphate 30 mg/kg (x) on tumour area under the curve at 90 seconds (AUC_{90}) shown as the ratio of central AUC: periphery AUC. Central pixels are more than 5.0 mm and peripheral pixels are less than 2.0 mm from the tumour edge. Data obtained from rats and shown as mean SEM. [6]
Background

- Two features likely to be an advantage in analyzing such data are (i) an automated approach, describing vascular parameters throughout the tumour and (ii) quantitative information about the dimensions of blood vessels and inter-vessel gaps.

- Structural Spectroscopy™ is a novel technique which enables the characterization of fine structure along the long axis of a right rectangular prism of material. A signal/distance plot, termed a “profile”, which is the measured variation in signal along the length of the prism is assessed using a novel combination of signal processing techniques, generating a Structural Frequency Analysis (SFA) spectrum. This SFA spectrum quantifies the relative or absolute amounts of repeating structures with various structural frequencies within that volume (structural frequency being the inverse of structural scale). [7]
Background

- Data is acquired from rectangular prisms located in the anatomical region and orientation of interest using a novel pulse sequence.

![Magnetic Resonance Prism Selective Sequence](image)

- The resulting one-dimensional signal profiles are then analyzed using customized signal processing algorithms to determine the spacing and/or size distribution of the anatomical elements of interest.

![Graphic user interface of Structural Spectroscopy analysis package](image)

**Figure 4:** OsteoTronix rectangular prism and MR Prism Selective Sequence. [7]

**Figure 5:** OsteoTronix graphic user interface of the Structural Spectroscopy analysis package. [7]
Background

• To assess the ability of the Structural Spectroscopy technique to characterise those structures and the validity of computer models of those structures physical phantoms are used. The physical phantoms are manufactured with known structures, usually of close packed spheres of polystyrene beads manufactured by Polysciences Inc..

Figure 6: Picture of the two OsteoTronix beads phantoms. A green labelled phantom contains microspheres of 180-212μm diameter. A yellow labelled phantom contains microspheres of 355-425μm diameter.
Aims

- The overall goal of this study is to have a tool which allow the automatic evaluation of structural changes, namely in tumour vasculature, due to the anticancer treatment.
- Firstly we will try to compare single and multi-phantom scans.
- Secondly, we will try to estimate the dimensions of vascular features in animal tumour models using a combination of structural spectroscopy analysis and gadolinium-based MR contrast agents.
Methods

• The phantom studies were carried out in a Varian 7T horizontal bore MR system.

• Firstly, each phantom was scanned individually in a 12mm $^1$H surface coil (m2m Imaging). A green labelled phantom contains microspheres of 180-212μm diameter. A yellow labelled phantom contains microspheres of 355-425μm diameter.

• Scout images were acquired and slices selected so the prism crossed the phantom.

• A one dimensional spin-echo pulse sequence was used to obtain spectroscopic data along the length of the 25x3x3 mm prism.

• The experiment was repeated with a single prism running through a water tube, green and yellow labelled phantoms (and air gaps) placed at the same time and level in the coil.
Methods

- *In vivo* measurements either before or after gadoteridol (0.1mmol/kg, ProHance, Bracco Imaging) were carried out on cdi nu/nu mice (n=5) implanted on the flank with HT29 human colon carcinoma cells. Measurements were taken 14-21 days after inoculation when the tumours were ~10mm in diameter. Mice had their tail vein cannulated and were restrained within a 33mm $^1$H quadrature volume coil (Rapid Biomedical GmbH) in the same Varian 7T horizontal bore MR system.

- Mice were anaesthetized with isofluorane/oxygen inhalation and kept warm using a warm air feedback system. Respiration rate was monitored.

- Scout images were acquired and slices selected to include tumour. A one dimensional spin-echo pulse sequence was used to obtain spectroscopic data along the length of a 13x1x1 mm prism, as shown in Figure 10 and 11 on the top. Images were collected before and 10 min after contrast agent injection. Parameters included TR = 500 ms, TE = 18 ms, prism dimensions 1x1x13 mm, gradient strength 6.5-8.7 G/cm, 1.6 ms sinc pulses, spectral width 100kHz, 512 data points, 512 averages. The distribution of spatial dimensions in the 1D spectra was determined using fineSA software (OsteoTronix Ltd, Swansea, UK). These are shown as structural frequency plots in Figure 10 and 11 on the bottom.
Figure 7: Structural spectroscopy in bead phantoms individually. In the lower graphs Probability Density function is displayed against structural frequency and structural scale.
Results – Multi-Phantom

Figure 9. Structural spectroscopy in both bead phantoms and water tube all at once. In the lower graphs Absolute raw data values are displayed against structural frequency and structural scale.
Results – *in vivo* before contrast agent

**Figure 11:** Structural spectroscopy in HT29 tumour before injection of gadoteridol (0.1 mmol kg\(^{-1}\)). Parameters included TR = 500 ms, TE = 18 ms, prism dimensions 1x1x13 mm, gradient strength 6.5-8.7 G/cm, 1.6 ms sinc pulses, spectral width 100kHz, 512 data points, 512 averages.
**Results – *in vivo* after contrast agent**

**Figure 11:** Structural spectroscopy in HT29 tumour after injection of gadoteridol (0.1 mmol kg). Parameters included TR = 500 ms, TE = 18 ms, prism dimensions 1x1x13 mm, gradient strength 6.5-8.7 G/cm, 1.6 ms sinc pulses, spectral width 100kHz, 512 data points, 512 averages.
Conclusions

• The phantom studies showed that the fineSA software can provide value information regarding several structures within the selected prism/ROI.

• There was a clear difference in the intensity of structural features seen in HT29 tumours grown in mice after injection of contrast agent (gadoteridol) compared to those before contrast. This is consistent with the highlighting of signals inside and adjacent to perfused tumour blood vessels, the features having spatial dimensions of 50-100 microns.
Future Work

• Future studies will include the use of the OsteoTronix package in other tumours (different cell lines);

• Different time points will be evaluated (to assess the time interval were the structures are still “visible” within the prism;

• Different drugs with known effects in blood flow and/or tumour vessels structures will be used to confirm the utility of OsteoTronix as an automatic tool to evaluate changes in tumour blood flow or structures as result of treatment.
References


