

## Primed Infusion of Gd.DTPA for Enhanced Imaging of Diffuse Lung Metastasis

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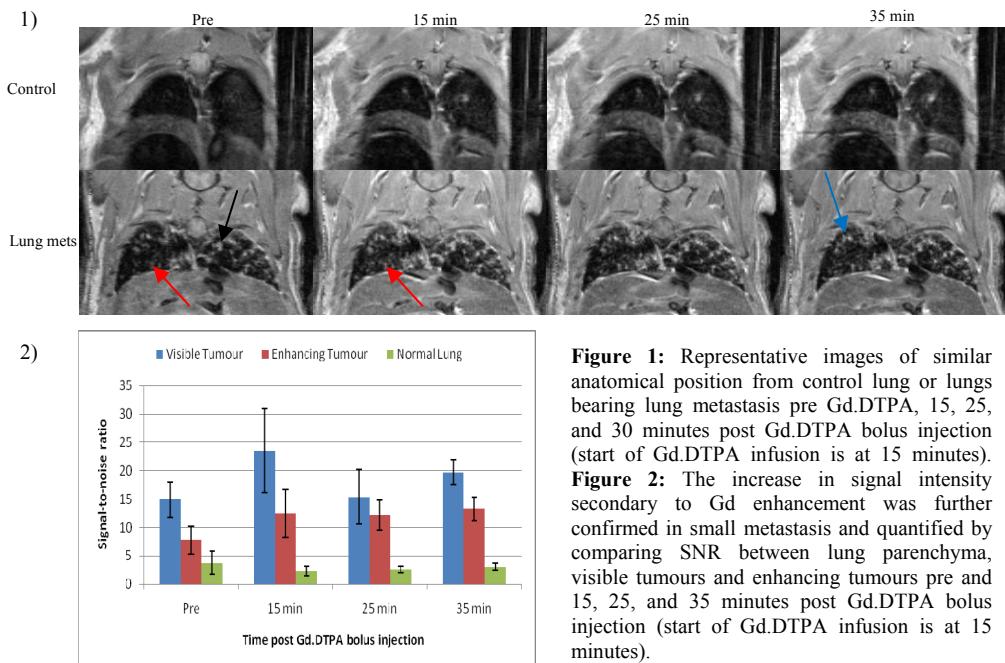
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**Introduction:** Gadolinium (Gd) enhancement has been widely used to investigate abnormal leakage from vasculature in many disease states including tumours. Gd(III)-chelates are small molecules with short half lives in blood. Gd leaks out of the capillaries and into the interstitial spaces, the rate of which can be used to give an indication of disease. Tumour blood vessels differ from vessels in normal tissues and are characterized by a chaotic network of tortuous, thin-walled leaky vessels<sup>1</sup> affording an increase in accumulation and retardation of wash out for contrast agents within viable tumour tissue by the enhanced permeability and retention (EPR) effect<sup>2</sup>. Blood clearance in normal tissues therefore leads to late gadolinium enhancement of tumour rim. However, the diagnosis of lung metastasis by MR imaging has proven complicated due to susceptibility and motion effects. Additionally, tumours are generally very heterogeneous and have varying degrees of viable to necrotic tissue, therefore small or largely necrotic metastases can be missed using Gd contrast. Studies by Tofts and Berkowitz (1994) showed that calculations of hybrid "primed infusions" from data derived from both bolus and continuous infusions in tissues of differing permeability have enhanced tissue uptake of Gd<sup>3</sup>. With continuous infusion, an equilibrium of Gd(III) concentration can be reached and sustained. This technique may be advantageous in lung metastasis imaging where equilibrium can provide continuous enhancement of the tumours with gated sequences. In this study, we have used primed infusions of the Gd(III) chelator Gd.DTPA (Magnevist) to assess enhancement of a lung metastasis model using a double gated spin echo sequence.

**Methods: Animal Model:** MDA-MB-231 breast cancer cells were grown in Dulbecco's Modified Eagles Media (DMEM) supplemented with 10% foetal bovine serum and 5% Penstrep. Cells were harvested and washed several times in PBS prior to injection. 6-8 week male NOD SCID gamma mice were anaesthetised with an isoflurane/O<sub>2</sub> mix, the tail vein cannulated and 2x10<sup>6</sup>/200μl PBS was administered by slow infusion. Mice were recovered and imaged at 30 days post injection (this was taken as the optimum time post administration as the lungs contain diffuse lung metastasis as seen by histology). Control mice were age and sex matched but did not receive an i.v. cannulation of cells.

**MRI:** Mice were anaesthetised with an isoflurane/O<sub>2</sub> mix and two i.p. lines were inserted (one bolus and one infusion line) prior to being placed into a quadrature <sup>1</sup>H volume coil and imaged using a 9.4T Varian VNMRS 20 cm horizontal-bore system (Varian Inc. Palo Alto, CA, USA). A spin-echo sequence double gated (respiration and cardiac) with the following parameters was used to assess T<sub>1</sub> enhancement, TR = as determined by the gating (remained constant throughout - typically 40 breaths/min and 550 beats/min), TE = 9.6 ms, FOV = 30 x 30 cm, averages = 1: matrix size = 256 x 128, slices = 10, thickness = 1 mm. Scan time was approximately 8 minutes. After the initial pre scan mice were given a bolus of 0.6mmol/kg Gd.DTPA, and 15 minutes after the bolus injection, an infusion of 0.05mmol/kg/min Gd.DTPA was started. Images were acquired directly after infusion and again at 25 and 35 minutes after bolus. Signal-to-noise ratios (SNR) were obtained from three regions of interest (ROI) on three consecutive slices for visible tumour (tumour that was apparent on pre scan - Fig 1 black arrow), enhancing tumour (area that enhanced at 15 min onwards that was not clear on pre scan - Fig 1 red arrows), and normal lung (ROI placed in control in the same anatomical place as visible tumours ROI), using the average signal intensity of each area, and the SD of the noise within the slice.

**Results:** On histology mice presented with metastasis throughout the lung that were heterogeneous in size with viable tissue. On baseline images only large tumours and some diffuse areas of increased signal intensity were discernable, which were not evident in control animals. 15 minutes after the initial Gd.DTPA i.p. bolus injection and directly after the start of the Gd.DTPA i.p. infusion (indicative of pure bolus enhancement) tumour tissue on images showed greater enhancement within the lung tissue as indicated by the red arrows in figure 1. At this time point the enhanced areas appear diffuse. However at the later time points of 25 and 35 minutes after the initial bolus the enhancement is sustained and appears more delineated and extensive (Fig1 blue arrow). At this point in the infusion we would expect Gd to reach equilibrium and it is clear that a high concentration of Gd is localised in tumours, compared to normal lung tissue, due to EPR. More lung metastases are visible post-contrast, indicating the ability of Gd to enhance small metastases. No enhancement of normal lung was observed in controls.



**Figure 1:** Representative images of similar anatomical position from control lung or lungs bearing lung metastasis pre Gd.DTPA, 15, 25, and 30 minutes post Gd.DTPA bolus injection (start of Gd.DTPA infusion is at 15 minutes).

**Figure 2:** The increase in signal intensity secondary to Gd enhancement was further confirmed in small metastasis and quantified by comparing SNR between lung parenchyma, visible tumours and enhancing tumours pre and 15, 25, and 35 minutes post Gd.DTPA bolus injection (start of Gd.DTPA infusion is at 15 minutes).

**References** 1) Fukumura *et al.* Am. J. Pathol. 1997;151;679. 2) Iyer AK *et al.* Drug Discovery Today. 2006;11;812. 3) Tofts and Berkowitz. Magn. Reson. Imag. 1994;12;81.

Signal enhancement was further confirmed and quantified by comparing SNR between normal lung, visible tumours, and enhancing tumours. Both the large tumours that were visible on baseline scans (visible tumours) and tumours that became visible due to enhancement from 15 minutes onwards (enhancing tumours) showed a clear increase in SNR from baseline. For enhancing tumours this appeared to be gradual over the time course, whereas for large tumours the 15 minute bolus SNR appeared to be maximal. This is most likely due to the size of the tumour rather than fluctuations in equilibrium, as the enhancing tumours appear to remain constant after 15 minutes. Large vascular tumours may accumulate high concentrations of Gd due to limited wash out by continuous infusion creating small susceptibility artefacts. There was no change in normal lung.

**Conclusions** These results show that primed infusions can enhance lung metastasis on double gated spin echo sequences affording clear delineation of small metastases as well as enhancing and improving the conspicuity of tumours from pre scan. This method may therefore have wide implications in the detecting of lung metastasis especially for early diagnosis of small metastases.