A Phase 1 Trial of SR4554 as a Non-Invasive Probe of Tumour Hypoxia detected by ^{19}F Magnetic Resonance Spectroscopy

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Introduction.

The degree of tumour oxygenation plays a vital role in the extent to which tumours respond to therapy. Currently the gold standard for measuring tumour hypoxia is use of Eppendorf polarographic microelectrodes. However these are invasive, require many tracks to achieve reliable results, and are only usable in relatively superficial tumours. SR4554 is a fluorinated 2-nitroimidazole, which has been designed as a non-invasive tumour hypoxia probe detectable by *in vivo* ^{19}F Magnetic Resonance Spectroscopy (MRS). It is reduced and bound in hypoxic cells but washes out of normoxic tissues. Hence a ratio of SR4554 signals acquired at early and later time points should give an indication of the degree of tumour hypoxia. The mechanism has been validated in pre-clinical systems (1) and is now undergoing Phase 1 Clinical Trials at the Royal Marsden Hospital and Institute of Cancer Research. The initial part of this study measured drug pharmacokinetics in patients, and also included a dose-escalation phase. The second (and continuing) part is investigating ^{19}F MRS signal visibility at 16 hours (approx 5 x $T_{1/2}$). In many tumours significant retention has been observed compared with plasma concentrations in the same patient.

Methods.

SR4554 was supplied by SRI International (and formulated by Cancer Research UK Formulation Unit at the University of Strathclyde) at a concentration of 200 mg/ml in 99 % dimethyl sulfoxy methoxide and 1% Tween 80. It was diluted in 0.9% normal saline and administered iv. Pharmacokinetics of parent SR4554 in plasma were measured using HPLC-UV (1). Prior to administration diagnostic MR images were obtained using standard clinical RF coils. In some patients diffusion and dynamic contrast-enhanced MRI were also acquired. MRS measurements were performed immediately after administration (MRS#1), and at a 2nd time point (MRS#2), using 5cm, 10 cm or 16cm diameter dual-tuned ^{1}H/^{19}F RF surface coils in a 1.5T Siemens Vision MR scanner. MR images were acquired using the TRUFISP sequence (TR = 6.32ms; TE=3ms). Automated shimming was performed using the method of Oliver Heid (2). ^{19}F MR data were acquired using a pulse-acquire pulse sequence with a short 1.28 ms tanh adiabatic RF pulse (to achieve uniform excitation within the field of view of the coil with minimum relaxation losses), TR = 1s and blocks of 512 averages. ^{1}H MRS data were acquired with the same sequence and coil, TR = 5s, NS = 8, to estimate drug concentration. In some patients CSI-localised ^{19}F data were also acquired at the first time point, using the same RF pulse. For the 2nd time point no additional localisation was used in order to maximise the number of quantifiable data sets. The retention index (RI) (%) was defined as (MRS#2/MRS#1) * 100. The "plasma RI" is the ratio of plasma concentrations at the same times. A reference sample (tetrafluorosuccinic acid) in the coil centre was used to verify coil operation.

18 patients have been enrolled in the study so far, 8 for the initial pharmacokinetic and dose-escalation phase (dose range 400 - 1600 mg/m^2) , and 10 for the second part investigating retention. Patients in this latter group had a median age of 55, performance status PS 0/1:1/9, and all received a dose of 1400 mg/m^2 over 30-60 minutes. Patients had histologically proven solid malignancy (> 3cm in size and < 4 cm from the skin surface) and were not on chemotherapy or radiotherapy at the time of the trial.

Results.

The results of the initial dose-escalation and plasma pharmacokinetic study have already been reported (3). This found the plasma $T_{1/2}$ of SR4554 to be approximately 3.3 hours, and the maximum tolerated dose to be 1400mg/m^2.

All 10 patients in the 2nd part of the study received SR4554 at 1400mg/m^2 (iv, over 30-60 mins) followed immediately by MRS#1. MRS#2 was acquired at ~16 hr post-infusion to detect ^{19}F signals indicative of tumour hypoxia following washout of parent SR4554. SR4554 was well tolerated. Toxicities observed were Grade 1 vomiting (n=1) and Grade 1 rash (n=1). PK studies of SR4554 showed: mean C_{max} = 112 ± 53 mg/L; mean T_{max} = 0.84hr ; mean $T_{1/2}$ = 3.9 ± 1.0 hr; mean V_{ss} = 30.8 L; clearance = 8.76 L/hr. There was excellent reproducibility of the ^{1}H MRS signals from tissue water between MRS#1 and MRS#2 (mean relative difference = 3.3 %; s.d. = 4.1 %). ^{19}F signals from SR4554 were seen in the early MRS scan in all 10 patients (e.g Fig 1a). At 16 hr SR4554 signals were above the threshold for detection in 7 patients (e.g. Fig 1b), yielding a mean RI of 11 ± 7% (range 0.6 to 21%) compared with 3.5 ± 2.4% in plasma (Fig 2). A paired t-test yields p = 0.007. MRS#2 data were below detection threshold in 2 patients, and not acquired in 1 patient. The tissue concentration at MRS#1, estimated from the ratio of 19F to 1H-water signals, are sometimes comparable with plasma levels and sometimes much lower. This may reflect delayed uptake in some tissues, but needs to be investigated further.

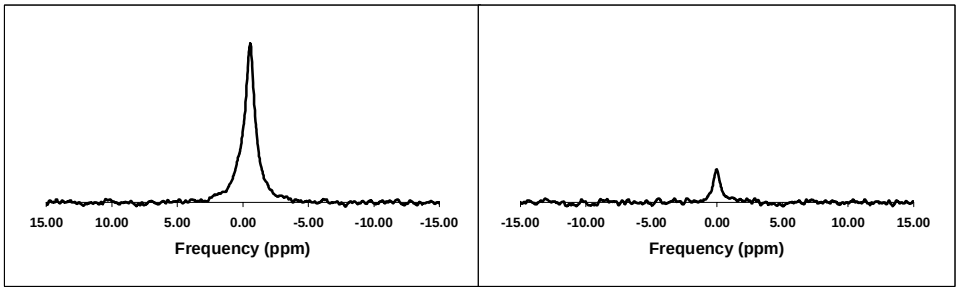
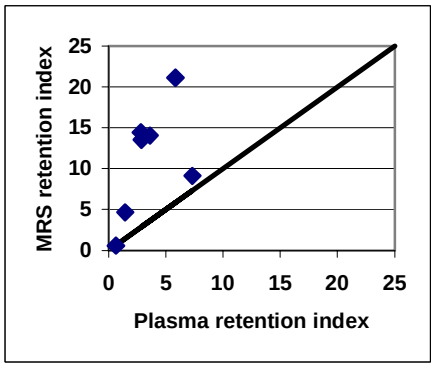


Fig 1. ^{19}F MRS data in Pat#14 at ~1 hour and ~16 hours after administration



Discussion and Conclusions. This trial to date has demonstrated that the study is feasible in the clinical context, with SR4554 visible by ^{19}F MRS. SR4554 is also observed to be retained in some tumours. The measured retention relative to plasma is in fact an underestimate as MRS#1 detects signal from all tissues, while it is expected that MRS#2 signal only arises from tumours. Localisation at both time points will address this issue. Despite very consistent plasma PK results, the observation of a range of values for RI relative to plasma supports the hypothesis that this may be a genuine indication of tumour hypoxia. Future studies will use localized MRS measurements and an independent marker of tumour hypoxia to further validate these findings.

References. (1) BM Seddon Clin Cancer Res **8:** 2323-2335 (2002). (2) OH Heid Proc ISMRM 1996, p363 (3) BM Seddon Clin Cancer Res. **9:** 5101 (2003).