Hyperpolarised combretastatins: potential bio-marker for vascular targeting of tumours.

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Introduction

Vascular targeting of tumours represents a proven complementary approach to conventional cancer therapy. However, effective clinical evaluation of new agents in this field requires the development of imaging bio-markers that can determine tissue pharmacokinetics (PK), early pharmacodynamic (PD) response and measures of resistant tumour phenotypes in early clinical trials. Vascular targeting agents, such as combretastatin A-4-phosphate (CA-4-P, Figure 1), are potentially useful in the treatment of tumours. Using hyperpolarisation (DNP), we aim to investigate the metabolic fate of CA-4-P in a BD9 rat tumour model.

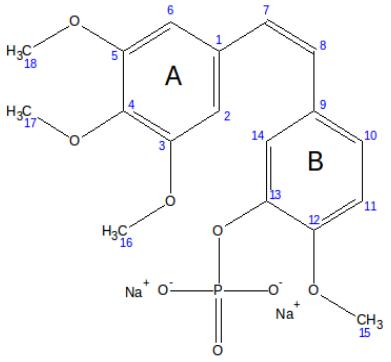

Figure 1: Combretastatin CA-4-P.

Method

Combretastatin CA-4-P was hyperpolarised using a HyperSense DNP polariser (Oxford Instruments Magnetic Resonance) operating at 1.40K. For ^{13}C hyperpolarisation 4.5mg (10.2mmol) of CA-4-P was dissolved in 200mL of 1:1 methanol-d4:DMSO-d6 and 15mM Finland radical (OIMR). The sample was polarised for ~6h and dissolved using 4mL of methanol. The ^{13}C spectrum was observed using a 90deg pulse-acquire sequence on a JEOL ECA400 spectrometer. To afford the best conditions for observing hyperpolarised combretastatins in vivo, it is favourable to ^{13}C label them at an appropriate position. A complete atom assignment (using 1D ^{1}H, 1D $^{13}C\{^{1}H\}$, ^{13}C HSQC, ^{13}C HMBC spectra) was performed using a 400MHz Bruker Avance III spectrometer, with a sample that consisted of 38mM CA-4-P dissolved in D_2O.

Results

The hyperpolarised ^{13}C NMR spectrum for natural abundance CA-4-P contains 6 hyperpolarised peaks, Figure 2. The measured ^{13}C T_1 and hyperpolarised signal to noise ratios (SNR) for CA-4-P, are displayed in table 1.

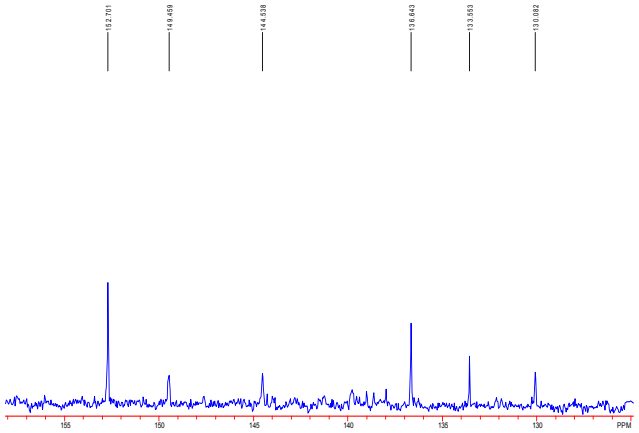

Figure 2: Hyperpolarised ^{13}C $\{^{1}H$, inverse gated$\}$ spectrum of CA-4-P.

Atom	δ, ppm	T1, s	SNR
1	133.5	2.5	7.5
3/5	152.7	5.6	27
4	136.6	12.3	18
9	130.1	2.6	7.5
12	149.5	6.9	6.8
13	144.5	8.3	7.2

Table 1: ^{13}C T_1 relaxation times and hyperpolarised SNR values for CA-4-P

Discussion

The measured T_1s show, as expected, that quaternary carbon atoms have the longest relaxation times: the longest being the well protected 4 position carbon atom, located on ring 'A'. The longest observed ^{13}C T_1 value for CA-4-P, 12.3s, may provide a small imaging window in which to observe the metabolism of CA-4-P. However, the 4 position is some distance away from the oxidative changes that occur within the molecule on ring 'B', and thus any chemical modification would produce only a modest to negligible chemical shift change at a ^{13}C labelled site. At present, polarisation of CA-4-P could be used for ^{13}C structural images to locate the presence of CA-4-P within the tumour. Strategies to increase the observed ^{13}C T_1 values could be to deuterate the molecule (which may change the kinetics of CA-4-P metabolism) or find combretastatin analogues (including ^{13}C acetyl tagging the molecule) to provide longer T_1 relaxation sites within the molecule.