Native T_1 is a Generic Imaging Biomarker of Response to Chemotherapy in Neuroblastoma

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Introduction

Neuroblastoma (NBL) is the most common extracranial solid tumour of childhood, accounting for between 7-10% of paediatric cancers. The proto-oncogene *MYCN* is amplified in 25% of NBL and is associated with high-risk disease, enhanced tumour angiogenesis and poor survival, making *MYCN* an attractive candidate for targeted therapeutics against NBL. A genetically-engineered mouse model for high risk, *MYCN*-amplified NBL has been previously generated, by directing expression of *MYCN* to the peripheral neural crest of transgenic mice (1). The resulting TH-*MYCN* mice develop tumours that mirror the genetic, pathophysiological and radiological characteristics of childhood NBL as well as its response to conventional chemotherapy, providing a promising platform for developmental therapeutics in NBL (2, 3). As part of a multi-parametric imaging study, we have evaluated the native MR relaxation parameters T_1 and T_2 as potential noninvasive biomarkers of treatment response of TH-*MYCN* NBL to three different classes of anti-cancer agent: specifically, the conventional cytotoxic drugs cyclophosphamide (CPM) and methotrexate (MTX), the VEGF signalling inhibitor cediranib, and the tubulin-binding agent N-acetyl colchinol (ZD6126).

Materials and Methods

TH-*MYCN* mice with abdominal tumours were identified by palpation and imaged prior to and following treatment (Table 1). ^{1}H MRI was performed on a 7T Bruker horizontal bore MicroImaging system, using a 3cm birdcage coil. Abdominal T_2 weighted RARE coronal images were acquired (FOV=4cm, matrix=128x128, 20 slices, 1mm thick, 4 averages, TE_{eff}=36ms, TR=5000ms, turbo factor=8). Anti-tumour activity of CPM, MTX, cediranib and ZD6126 were assessed by quantification of the tumour volume, using segmentation of ROIs drawn on each tumour-bearing slice. Native T_1 and T_2 were quantified from a single 1mm thick axial slice obtained by an inversion recovery (IR)-trueFISP sequence (FOV=3cm, matrix=128x96, 1 slice, 1mm thick, 8 averages, TI=25-1450ms, 50 inversion times, TE=1.2ms, TR=2.5ms, scan TR=10s, 8 segments). The IR-trueFISP data were fitted voxelwise using in-house software, yielding quantitative maps of T_1 and T_2. MR imaging biomarkers of native T_1, T_2 and tumour volume were compared and validated with uptake of the perfusion marker Hoechst 33342 assessed using fluorescence microscopy, for the extent and distribution of functional tumour blood vessels, and haematoxylin and eosin staining for necrosis. Any significant changes in tumour volume, quantitative MR or histological parameters with treatment were identified using Student's 2-tailed paired t-test, with a 5% level of significance.

Table 1. Summary of drug doses and imaging schedules

Drug	Control 1	Control 2	CPM	MTX	cediranib	ZD6126
Dose regimen	-	-	25 mg/kg i.p.	100 mg/kg i.p	6 mg/kg p.o. daily over 2 days	200mg/kg i.p.
Post treatment MRI	48h	4 days	48h	48h	24h after final dose	24h
Cohort size	10	4	3	3	10	7

Results and Discussion

Changes in native T_1 and tumour volume with treatment are summarised in Figure 1. No significant effects on T_2 were found. In the control cohorts, tumour progression over 48 hours and 4 days was associated with no significant change in T_1. A single dose of CPM, the current standard of care for childhood NBL, caused a significant reduction of tumour volume at 48 hours. Treatment of TH-*MYCN* NBL with CPM leads to extensive cell death, which occurs primarily through apoptosis (4). This response was associated with a highly significant reduction in native T_1. Treatment with MTX resulted in no significant change in either tumour volume or T_1. The differential response of TH-*MYCN* tumour to CPM and MTX thus mirrors the differential sensitivity of childhood NBL to these cytotoxic agents.

Given the hypervascular nature of NBL (3), their response to anti-vascular therapies was investigated. Treatment with the pan VEGF receptor inhibitor cediranib resulted in significant anti-tumour activity and reduction in T_1 at 48 hours. Furthermore, this response was associated with a significant reduction in Hoechst 33342 uptake compared to control, but no difference in necrosis. Treatment with the colchicine derivative ZD6126 resulted in significant and unprecedented anti-tumour activity at 24 hours, and which was also associated with a significant reduction in native T_1 (Figure 2). Histology revealed a significant reduction in Hoechst 33342 uptake, and extensive tumour necrosis. The acute 40% antitumour activity following treatment with ZD6126 is presumably a consequence of both its established vascular disrupting effects on proliferating endothelial cells lining tumour blood vessels, and direct cytotoxic effects on neuroblasts through binding to the colchicine binding site of tubulin (5).

Collectively, these data show a systematic reduction of native T_1 in the TH-*MYCN* model with successful chemotherapy. The CPM and ZD6126-induced reduction in T_1 is consistent with a histologically confirmed reduction in cell density and increased extravascular space following cytotoxic therapy (6). The reduction of T_1 following treatment with cediranib may be attributable to the resolution of oedema caused by reduced vascular permeability, a response observed following treatment with other VEGF signalling inhibitors (7).

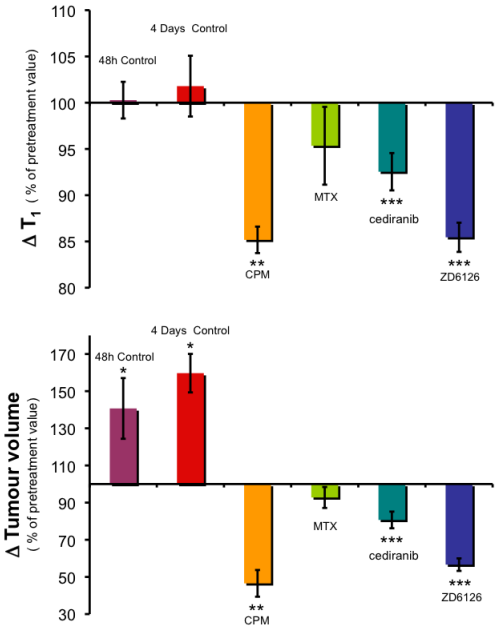

Figure 1. Change in T_1 and tumour volume of TH-*MYCN* mice following chemotherapy. (*p<0.05, **p<0.01, ***p<0.005, Student's 2-tailed paired t-test)

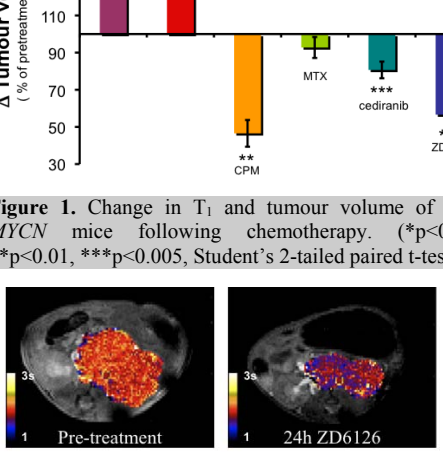

Figure 2. Representative parametric T_1 maps of a tumor from a TH-*MYCN* mouse acquired a) before and b) 24 hours after treatment with 200mg/kg ZD6126.

Conclusions

The accurate quantification of T_1 affords a generic noninvasive biomarker for chemotherapy-mediated cell death in the TH-*MYCN* model, reinforcing recent pre-clinical and clinical studies which demonstrate native T_1 as a generic imaging biomarker of cell viability (6,7). The high sensitivity of T_1 will accelerate the pre-clinical evaluation of novel therapeutics for childhood neuroblastoma in this model. Quantitation of native T_1 could be easily incorporated into DCE-MRI protocols used in clinical trials.

References. (1) Weiss et *al., EMBO J* (1997), **(2)** Jamin et *al.*, *Proc ISMRM* (2010), **(3)** Chesler et *al.*, *Cancer Res* (2008), **(4)** Chesler et *al.*, *Neoplasia* (2008), **(5)** Meany et *al., Pediatr Blood Cancer* (2010) **(6)** McSheehy et *al.*, Clin Can Res (2010) **(7)** O'Connor et *al*., *Clin Cancer Res* (2009).

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