Native T1 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 T1 and T2 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 colchicine (ZD6126).

Materials and Methods

TH-MYCn mice with abdominal tumours were identified by palpation and imaged prior to and following treatment (Table 1). 1H MRI was performed on a 7T Bruker horizontal bore MicroImaging system, using a 3cm birdcage coil. Abdominal T1 weighted RARE coronal images were acquired (FOV=4cm, matrix=128x128, 20 slices, 1mm thick, 4 averages, TR=300ms, TE=50ms). 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 T1 and T2 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 T1 and T2. MR imaging biomarkers of native T1, T2 and tumour volume were compared and validated with uptake of the perfusion marker Hoescht 33342 assessed using fluorescence microscopy, for the extent and distribution of functional tumour blood vessels, and haematocrit 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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Control</th>
<th>Control 2</th>
<th>CPM</th>
<th>MTX</th>
<th>cediranib</th>
<th>ZD6126</th>
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</thead>
<tbody>
<tr>
<td>Dose regimen</td>
<td></td>
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<tr>
<td>Post treatment MRI</td>
<td>48h</td>
<td>4 days</td>
<td>25 mg/kg i.p.</td>
<td>100 mg/kg i.p</td>
<td>6 mg/kg p.o. daily over 2 days</td>
<td>200mg/kg i.p.</td>
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<tr>
<td>Cohort size</td>
<td>10</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>7</td>
</tr>
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</table>

Results and Discussion

Changes in native T1 and tumour volume with treatment are summarised in Figure 1. No significant effects on T1 were found. In the control cohorts, tumour progression over 48 hours and 4 days was associated with no significant change in T1. 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 T1. Treatment with MTX resulted in no significant change in either tumour volume or T1. 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 T1 at 48 hours. Furthermore, this response was associated with a significant reduction in Hoescht 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 was also associated with a significant reduction in native T1 (Figure 2). Histology revealed a significant reduction in Hoescht 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 T1 in the TH-MYCn model with successful chemotherapy. The CPM and ZD6126-induced reduction in T1 is consistent with a histologically confirmed reduction in cell density and increased extracellular space following cytotoxic therapy (6). The reduction of T1 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).

Conclusions

The accurate quantification of T1 affords a generic noninvasive biomarker for chemotherapy-mediated cell death in the TH-MYCn model, reforming recent pre-clinical and clinical studies which demonstrate native T1 as a generic imaging biomarker of cell viability (6,7). The high sensitivity of T1 will accelerate the pre-clinical evaluation of novel therapeutics for childhood neuroblastoma in this model. Quantification of native T1 could be easily incorporated into DCE-MRI protocols used in clinical trials.


Acknowledgements. We acknowledge the support received from the CRUK and EPSRC Cancer Imaging Centre in association with the MRC and Department of Health (England) grant C1060/A10334, NHS funding to the NHR Biomedical Research Centre, AstraZeneca and the Royal Society. Cediranib was provided by AstraZeneca and ZD6126 was provided by Angiogene.