Early accumulation of 1H MRS detected lipids and lactate in rat 9L glioma to anti-angiogenic treatment

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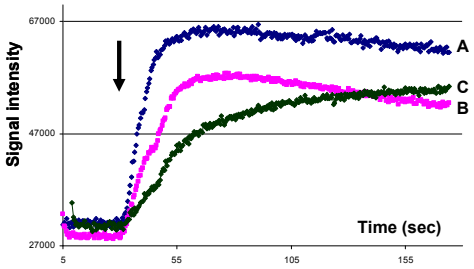

Fig. 1. T_1-weighted FLASH signal time course following Magnevist bolus (indicated by an arrow) in a 9L glioma. Trace A from untreated, trace B from animal at B20-4.1.1 treatment day 3 and trace C at treatment day 7.

INTRODUCTION

Clinical experience from anti-angiogenic treatment with an anti-VEGF antibody (bevacizumab) shows a median progression free survival of 16 weeks with a 6 month progression free survival of 29% for glioblastoma multiform patients who were previously treated with combined standard radiotherapy and temozolomide chemotherapy [1]. Although the response rate is favourable, in most cases the tumour eventually recurs or continues to progress. Thus, it would be vital to have imaging surrogate markers that reveal responders before tumour volume changes. There is a good deal of evidence to show that both vascular permeability and blood flow decrease, as determined by dynamic susceptibility contrast and arterial spin labeling MRI, respectively, in patients responding to anti-angiogenic treatment [2,3]. In the current study a rodent anti-VEGF-antibody, B20-4.1.1, to mimic clinical bevacizumab treatment in an orthotopic 9L glioma of the rat and multi-modal MRI and ^{1}H MRS were used to sequentially monitor tumour status during treatment with B20-4.1.1 alone or in combination with a conventional anti-cancer drug, irinotecan.

METHODS

Male Fisher rats were inoculated with $5x10^4$ 9L tumour cells in 10 µl into the left hemisphere. Tumour bearing rats (n=5) were treated with B20-4.1.1 (5 mg/kg ip 3 times a week) without (n=3) or with irinotecan (50mg/kg every two weeks) starting on day 15 after inoculation. For MR, rats were anaesthetised with isofluorane (1-1.5% in 70:30 O_2:N_2) through a nose cone, secured to an animal holder with tooth and ear bars and scanned using a 7T Varian Inova system with a surface coil for transmit/receive. Body temperature was maintained with a thermostated water circulating heating element during experiments. RARE sequence was used for acquisition of T_2-weighted images (TR=3s, TE=60ms, 256x128 pixels, FOV 3x3 cm^2, 1mm slice), standard Hahn echo sequence was used to acquire T_1- weighted images (TR=700ms, TE=9ms, 256x128 pixels, 3x3 cm^2, 1 mm slice). The trace of the diffusion tensor (D_{av}) was quantified using a SE sequence incorporating four bipolar gradients along each axis [4]with three b-values ranging from 0 to 1370 s/mm^2 (TR=1.5 s, TE=55 ms). FLASH with 1.75 frames/s (TE 1.8 ms, 128x64 pixels, FOV 3x3cm^2, flip angle 25°) from the center of tumour mass was used to collect data for T_1-weighted signal following a rapid gadodiamine-bolus (0.2 mmol/kg iv). LASER technique was used for single-voxel ^{1}H MRS from main bulk of tumour stroma with TR=2.5 s and TE=27 ms [5]. MR images were analysed for D_{av}, tumour volume and Gd-signal enhancement using Aedes (www.aedes.uku.fi.) under Matlab platform. TARQUIN [6] was used for spectral data analyses expressed as proton contribution corrected ratios to total creatine. Values shown are means ± SD.

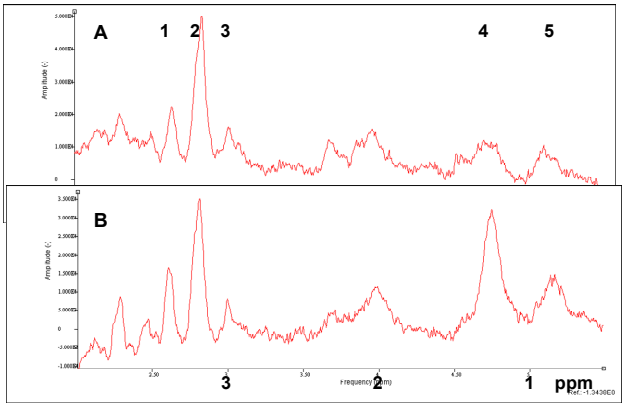

Fig. 2. ^{1}H MR spectra from untreated (A) and B20-4.1.1 treated (day 3) 9L Glioma. Peaks are assigned as follows: 1: taurine, 2: Choline containing compounds + taurine, 3: total creatine, 4: lactate + 1.3Lipids and 5: 0.9Lipids

RESULTS

Untreated rats died 22.3±1.5 after inoculation, whereas treated rats survived for 31.2±10.8 days. Neither D_{av} nor the rate of T_2-volume expansion were different between untreated and untreated rats (Table 1). There was a tendency towards a slower expansion rate of T_1-post-Gd volume upon treatment (Table 1). Time course of T_1-weighted signal following Magnevist bolus was severely slowed down upon B20-4.1.1 treatment (Fig. 1). Time-to-maximum prolonged nearly 3-fold in B20-4.1.1 treated rats during the first week of treatment; this effect was chiefly due to the antibody, because drug combination showed no further prolongation of the T_1-signal kinetics (Table 1). ^{1}H MRS revealed a strong increase in peaks centered at 1.3 and 0.9 ppm in a treated tumour as early as day 3 (Fig. 2). TARQUIN analysis of *in vivo* spectra revealed substantial elevations Lac/Cr, 1.3Lip/Cr and 0.9Lip/Cr ratios, that paralleled the observed slow down of T_1-signal kinetics following Magnevist (Table 1). Cholines (CCM/Cr) did not change.

Table 1. MR variables in 9L gliomas following treatment with B20-antibody with and without irinotecan.

VARIABLE	UNTREATED	ALL TREATED (Day 3)	ALL TREATED (Day 7)	B20-TREATED (Day 3)	B20-TREATED (Day 7)
D_{av} (10^{-3} mm^2/s)	0.95±0.05	0.98±0.07	0.89±0.15	1.02±0.06	0.85±0.20
T1-vol exp (mm^2/day)	16.8±5.0	Nd	8.6±6.0	Nd	9.7±5.4
T2-vol exp (mm^2/day)	16.2±8.0	Nd	13.4±4.1	Nd	12.9±5.2
Time-to-max (sec)	11.8±3.6	23.8±11.8*	37.8±12.3*	19.3±9.6*	30.2±8.3*
CCM/Cr	1.13±0.51	1.31±0.39	1.05±0.24	1.35±0.53	1.20±0.12
Lac/Cr	1.78±0.78	3.05±0.91*	4.49±3.02*	3.49±0.93*	5.46±3.83*
1.3Lip/Cr	1.34±0.98	6.03±3.30*	8.98±7.00*	7.52±3.69*	11.69±8.40*
0.9Lip/Cr	1.00±0.62	2.43±0.63*	2.93±1.55*	2.64±0.79*	3.57±1.80*

CONCLUSIONS

The current results indicate that the anti-VEGF-antibody exerts beneficial effect on clinical behaviour of 9L rodent glioma. B20-4.1.1 slows down Gd-leakage to the tumour, an effect that is likely to be due to combination of reduced perfusion and vascular leakiness. ^{1}H MRS shows large increase both in saturated lipids and lactate during early B20-4.1.1 treatment. Taking into account unchanged diffusion in tumour stroma upon treatment, the ^{1}H MRS alterations indicate severed hypoxia associated with cell growth arrest and/or induction of apoptosis, but no true cell eradication [7]. Thus, combining ^{1}H MRS with MRI techniques probing diffusion and vascular functions provides a fuller picture of anti-angiogenic treatment effects and assists detection of favourable response at early phase of therapy.

ACKNOWLEDGEMENTS

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