Statistical power of two quantitative biomarkers for analysis of DCE-MRI studies of agents targeting tumour vasculature

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Introduction

Tumour vasculature presents an attractive target for therapy. There are two major pharmacological classes of agents, both cytostatic in effect when used as single agents[4]: vascular disrupting agents (VDAs) selectively attack existing tumour vasculature, while anti-angiogenic agents restrict the growth of new blood vessels. Dynamic contrast enhanced MRI is extremely useful in the evaluation of agents of these types. There is no definite consensus on the analysis of DCE-MRI, but workshops for both CRUK[1] and the NCI[2,3] have recommended equally two biomarkers based on such analyses: compartmental modelling[5,6] , which gives a transfer constant K^{trans} for contrast agent into the extracellular extravascular space (EES); and initial area under the gadolinium-time curve (IAUGC)[7],which makes no assumptions about the underlying physiology.

If one biomarker has higher statistical power, its use could enable studies with fewer patients, detect responses earlier in dose-escalation studies, and allow reduced animal usage in pre-clinical studies. The two major pharmacological classes may show different effects on the biomarker distributions. This work therefore examines the relative statistical power of the two biomarkers for examples of both pharmacological classes: the VDA ZD6126, a tubulin binding agent; and ZD6474 (ZACTIMA), an anti-angiogenic signalling inhibitor that selectively targets key pathways in tumour growth.

Methods

In order to avoid unnecessary use of animals, or human studies which could not easily be justified ethically, the study was performed by further analysis of data from previous pre-clinical dose-response and regrowth studies. The original studies were carried out at two sites using the same protocols and identical Varian Unity Inova MRI scanners equipped with 4.7T horizontal-bore magnets. Briefly, baseline T1 values were acquired before contrast administration. Four contiguous slices were obtained through the tumour and one abdominal slice was acquired to provide an IAUGC reference from paraspinal muscle. The matrix size was128×128, TR=120ms, TE=10ms, giving a time resolution of 15.4s. Five image sets were acquired prior to and 40 sets after bolus injection of 0.1 mmol/kg GdDTPA. All animals were scanned both pre- and post-treatment with agent or vehicle. Studies on the anti-angiogenic agent[8] were carried out on PC3 tumours grown in female Swiss athymic mice, at doses of 0, 12.5, 25, 50 or 100 mg kg^{-1}/day 2 and 24 hours prior to the second DCE-MRI scan. Studies on the VDA[9] were carried out on GH3 prolactinomas grown in female Wistar Furth rats, at doses of 0, 12.5, 25 or 50 mg kg^{-1} 24 hours prior to the second DCE-MRI scan. All non-vehicle doses are known from other work to elicit a biologic response from the tumours. Dose-response and efficacy were previously demonstrated in both models by IAUGC analysis (VDA) and Tofts' K^{trans} (signalling inhibitor) and histology. Tofts K^{trans} and IAUGC analysis were performed in software custom-written in Matlab. Standard AIFs were used for mouse and rat. IAUGC values were calculated at 60 and 150 seconds post contrast injection in accordance with workshop recommendations. One-tailed paired t-tests were used to compare values pre- and post-treatment. The relative statistical powers of the methods were assessed by comparing the p-values at each dose of each agent, and by using the t-statistic to estimate the minimum number of animals required for a significant result at each dose.

Results

Significant changes were observed in all three biomarkers K^{trans}, IAUGC60 and IAUGC150 at the higher dose levels. For the VDA, there was a large increase in the number of voxels which could not be fitted by the Tofts model after treatment, especially at the highest dose. All three biomarkers were reduced post treatment at all dose levels, with smaller p values at higher dose levels. The results are summarised in Table 1 as p-values for each dose level. The two highest doses of the signalling inhibitor and the highest dose of the VDA gave consistently highly significant results ($p<0.005$). At lower doses, the different biomarkers gave variable results; for example, for the VDA at 25 mg/kg, analysis of K^{trans} gave p=0.0029, while analysis of the same data using the IAUGC150 biomarker gave p=0.09. Hence, no single biomarker was more consistently sensitive to the effects of the agents at doses well below maximum biological effect. Table 2 presents estimates of the number of animals required to obtain significance at each dose level of both agents.

	Signalling inhibitor ZD6474 dose (mg/kg)				
	Vehicle	12.5	25	50	100
IAUGC 60	0.89	**0.043**	0.14	**0.003**	**0.005**
IAUGC 150	0.19	**0.03**	**0.03**	**0.003**	**0.001**
K^{trans}	0.61	0.13	0.15	**0.0002**	**0.0002**
	VDA ZD6126 dose (mg/kg)				
	Vehicle	12.5	25	50	
IAUGC 60	0.25	0.098	**0.003**	**0.0006**	
IAUGC 150	0.21	0.09	0.09	**0.002**	
K^{trans}	0.23	**0.039**	**0.003**	**0.0001**	

Table 1. p-values for 1-tailed paired t-tests for each dose group pre and post treatment with the two vascular targeting agents

	ZD6474 dose (mg/kg)			ZD6126 dose (mg/kg)		
	25	50	100	12.5	25	50
IAUGC 60	17	6	7	10	4	4
IAUGC 150	7	6	6	10	10	6
K^{trans}	19	5	5	6	4	4

Table 2. Estimated numbers of animals required to obtain significance at each dose level for the VDA ZD6126 and the anti-angiogenic signalling inhibitor ZD6474

Conclusions

At higher doses where the biological effects of the agents are greatest, K^{trans} and IAUGC at 60 and 150 seconds all give highly significant p values. At lower doses, where other biomarkers such as histological necrosis demonstrate that the agents have detectable biological effects, none of the DCE-MRI biomarkers is consistently more sensitive to the effects of the agents. There is therefore no compelling reason to prefer any biomarker for its statistical power. IAUGC may be recommended as it is very simple to calculate and does not suffer from fit failures in regions of low flow, as can be the case with K^{trans}.

References

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