Early effects of combretastatine A-4-P on rat bladder tumor heterografts assessed by combined carbogen based fMRI and T2 mapping

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Introduction

Tubulin disrupting agents like combretastatin A-4-P (CA-4-P) exhibit potent anti-vascular activity in malignant tumors, and are currently in preclinical and clinical development for antivascular tumor therapy [1]. We recently showed on a rat bladder tumor model, that CA-4-P reduced, as early as about 35 min post-administration, the tumor fraction with T2*-weighted (T2*w) signal enhancement under carbogen breathing. This early effect was confirmed by tumor growth rate reduction measured at 24h. [2]. In the current preliminary study we combined carbogen based fMRI and classical T2 mapping to assess effects of CA-4-P on tumor vasculature and necrosis.

Subjects/methods

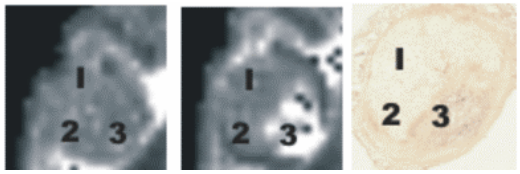
NBTII-FGF1 rat bladder tumors (overexpressing FGF1) were implanted subcutaneously in the flank of female nude mice (n=4). CA-4-P was i.p. injected (100mg/kg in 0.02ml/g NaCl 0.9%). Experiments were performed on a Bruker Biospec (4.7T). MRI acquisitions were synchronized with respiratory motion. The imaging protocol comprised a MSME sequence for quantitative T2 measurements (FOV=(3cm)^2, matrix 128x128, Slth 1mm, TR/TEmin=490/9.6msec, 8 echoes), and a fast gradient echo sequence, allowing an entire T2*w image acquisition per respiratory cycle, to perform "BOLD"-type imaging (FOV=(3cm)^2, matrix 128x80, Slth 1mm, TR/TE=26.6/16.3ms, α=30º). 80 sets of 15 contiguous slices comprising the tumor were acquired during the following protocol of breathing gas administration: image set 1-7: air, image set 8-48: carbogen, image set 49-80: air. T2*w images and T2 maps were acquired before, about 35min and 24 hours after CA-4-P injection. Subsequently, tumors were excised and examined by histology (hematoxilin and antibody anti FVIII staining). T2 maps were calculated by mono-exponential fitting. Tumor necrosis was estimated on T2 maps after definition of a T2 threshold (93 ms) which yielded, for one arbitrarily chosen tumor, a necrotic fraction similar to that determined by histology. The time-course of signal intensity (SI) variation I/I0 in response to carbogen breathing measured in ROIs defined on T2 maps, and tumor fractions with signal enhancements under carbogen breathing (T+) were determined by counting corresponding voxels in manually defined ROIs covering the whole tumor [3].

Results

We observed a good correlation between necrotic fractions determined by histology and by T2 maps (r=0.99, p=0.003). For all tumors, necrosis increased 24 hours after CA-4-P administration (Fig. 1). With fMRI we observed at 35 min a T+ decrease for 3 tumors and no T+ modification for one tumor.

T2 modifications were also observed as early as 35 min post CA-4-P. In all tumors, three regions with different T2 evolutions could be identified. Region 1: T2 decreased at 35 min after CA-4-P administration and recovered at 24h. Region 2: T2 did not change after CA-4-P administration. Region 3: T2 increased at 35 min and further at 24h after CA-4-P administration. Region 3 corresponded to necrosis as confirmed by histology (Fig 1).

Interestingly, regions 1 and 3 showing specific modifications on T2 maps showed also systematically different behaviours on fMR images upon carbogen breathing. Region 1 : Initial T2*w SI increase disappeared at 35 min (Fig. 2 A), and 24h post CA-4-P. Region 3 : T2*w SI increased before and decreased at 35 min (Fig. 2 B), and 24h post-CA-4-P.



A B C

Figure 1: T2 maps before (A) and 24h after (B) CA-4-P administration and the corresponding histological slice (C). Numbers indicate regions with different T2 evolutions.

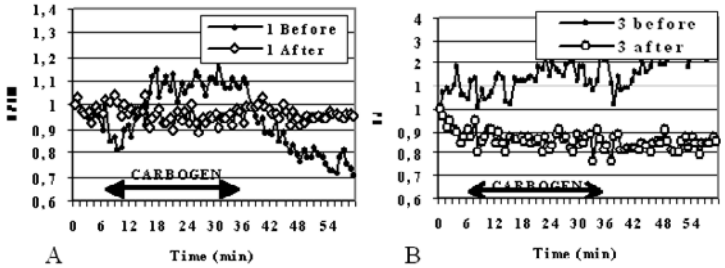


Figure 2: T2*w SI modification upon carbogen breathing before and at 35 min after CA-4-P administration observed in regions 1 (A) and 3 (B) of Fig. 1

Discussion/Conclusion

An early effect of CA-4-P was not only observed by carbogen based fMRI, as already described before [2], but also on classical T2 maps (without carbogen breathing). The early T2 decrease (region 1) might be due to increase of deoxyhemoglobin as a consequence of vascular disruption, which should be also responsible for the disappearance of carbogen response in this region. Early responses were observed also in region 3, which was found to be necrotic at 24h. Explanation of both early response patterns, T2 increase and T2*w signal decrease upon carbogen breathing will need further investigation.

Our results suggest, for the first time, that a CA-4-P effect can be detected very early even by classical T2 mapping at 4.7T. The predictive value of the observed early modifications for therapy outcome will have to be determined. T2 mapping alone or in combination with carbogen based fMRI may become a tool for antivascular therapy follow-up without the need of exogenous contrast agents.

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