Effect of Carbogen Breathing on a Chemically Induced Hepatocarcinoma Model in Mice

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Introduction

Multiparametric non-invasive follow-up of tumor development by combined morphological and functional MRI should become a powerful tool for prognosis and evaluation of the new therapies targeting neo-angiogenesis. At a preclinical state, studies on mouse models of chemically induced slowly developping orthotopic tumors are necessary and complementary to the frequently performed studies on subcutaneously implanted rapidly growing tumors as there may be important differences in tumor vascularization. We recently performed a preliminary investigation of an induced multifocal liver tumor model in mice characterized by the presence of two tumor types (avascular cholangioma and vascularized hepatocellular carcinoma (HCC)) showing, in particular and for the first time on this model, the feasibility of measuring a carbogen effect [1]. The purpose of the present study was to characterize in more detail and during longitudinal follow-up the tumor response to carbogen breathing.

Methods

Liver tumors were chemically induced in XVIInc/z female mice (n=13) by three subcutaneous injections of 5,9-dimethyl-dibenzo-[c,g]-carbazole, an organ specific carcinogen for mouse liver [2]. MRI studies were performed at different stages of tumor development beginning at week 20 after induction. Mice were anaesthetized with isoflurane (2%), inserted in a cradle in supine position, the body temperature being kept constant by a warm water carpet. MRI acquisitions were synchronized with respiratory motion using a home-built high sensitive respiratory trigger. Experiments were performed at 4.7 Tesla on a Bruker Biospec using a slotted cylinder type probe (d=44mm). The imaging protocol comprised a fast spin echo sequence ("3D-RARE") for high resolution screening of the whole liver and measurement of growth curves (FOV 3x3x2.5 cm^3, matrix 256x256x50, TR about 2sec (resp. rate), TEw=41msec, RARE factor 16), a MSME sequence for T2 measurements (FOV=$(3cm)^2$, matrix 128x128, Slth 1mm, TR/TEmin=490/9.6msec, 8 echoes) and a fast gradient echo sequence ("GEFI") allowing an entire T2* weighted image acquisition per respiratory cycle to perform "FLOOD"-type imaging [3] (FOV=$(3cm)^2$, matrix 128x80, Slth 1mm, TR/TE=26.6/16.3msec, pulse angle 30deg). 80 sets of 15 slices covering the whole liver were acquired during the following protocol of breathing gas administration: image set 1-7: 20%O_2/80%N_2, image set 8-48: carbogen (95%O_2/5%CO_2; 2l/min), image set 49-80: 20%O_2/80%N_2. Mean signal intensity variation was measured on manually defined ROIs covering the whole tumor on an axial slice passing through the tumor center. At the end of MRI experiments (when tumor diameter reached about 5mm) the mice were sacrificed and the livers removed for macroscopic and histological examination (tumor vessels were highlighted by immunocytochemical staining to detect factor VIII-related antigen (Dako) following hematoxylin staining). Care was taken that slice orientation matched MRI slice orientation as well as possible.

Results

A total of 50 HCCs could be detected (T_2(HCC)=40msec, T_2(liver)=30msec) and were followed over a mean period of 6 weeks at a frequency of 1 measurement per week. They were found to grow quickly (doubling time down to two weeks). The tumors were well vascularized and macroscopic vessels could often be seen at the tumor surface at autopsy. In most of them a significant response to carbogen breathing could be detected, however the timecourse of intensity variation was found to depend on the individual tumors. After inspection of the individual response curves 4 types could be distinguished: 74% of the tumors showed signal enhancement starting shortly after onset of carbogen breathing (type 1, fig. 1), 8% showed a delayed signal increase starting at the end of the carbogen breathing period and lasting after breathing gas returned to air (type 2), 10% showed little increase (max 4%) or no response (type 3), and 8% showed a decrease of signal intensity (type 4). This decrease could be attributed to the presence of necrotic regions by comparison with the observation (in a separate experiment) of signal decline in a well identified (MRI, histology) large necrotic region.

It has to be noted that, during the follow-up of a given tumor, the same type of timecourse was always observed (fig. 2) except for tumors with necrotic regions appearing in the ROI at the end of the follow-up period.

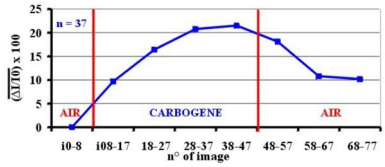

Figure 1 : Type 1 mean response to carbogen breathing

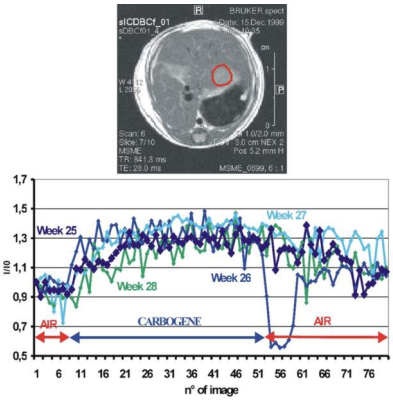

Figure 2 : Exemple of longitudinal study of HCC response (type 1) to carbogen breathing

Discussion

These results first confirm the possibility of completely non-invasive follow-up of vascularization dependent signal change as well as morphological parameters on this model which should make it suitable for evaluation of anti-angiogenic therapy. The majority of tumors exhibit a "type 1" response compatible with an increase of oxyHb/deoxyHb ratio in a hypoxic tumor which could possibly serve as a selection criterion for inclusion in a treated group. 8% of the HCCs showed a delayed response (type 2), perhaps the vascular architecture delayed the carbogen arrival (tortuosity of the vessels), perhaps the tumor environment partly captivated the carbogen ("steal effect"). Finally tumors with type 3 response may be not enough hypoxic to have their oxyHb/deoxyHb sufficiently modified or their vascular network isn't functional or they are very few vascularized. So far, it may be hypothesized that evolution towards a "type 4" response, related to necrosis, could have prognostic value for therapeutic monitoring. Studies comprising comparison with histological data and perfusion measurements in order to explain the different response types are currently in progress.

References

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Acknowledgement

This work was supported by a postdoctoral grant (CDT) from the "Association pour la Recherche contre le Cancer (ARC)"