Characterization of Different Drug Treatments Efficacy in morris hepatocellular Carcinoma (HCC) Bearing Rat with a Desktop MRI

Luca Venturi¹, Luigi Miragoli², Claudia Cabella², Lara Caminiti², Fabio Tedoldi² and Silvio Aime¹.

¹ Dipartimento di Fisica Sperimentale, Università degli Studi di Torino, Via Giuria 1, 10125, Torino, (TO), Italy
² Centro Ricerche Bracco, Bracco Imaging Spa, Via Ribes 5, 10010, Colleretto Giacosa, (TO), Italy Contact: luca.venturi@unito.it

Introduction

New Bruker icon desktop MRI scanner –Bruker BioSpin MRI- was employed for the therapeutic follow-up of 15 Morris hepatoma bearing rats monitored upon treatment with 2 different regimes.

A group of 6 animals was treated with Sorafenib, a multikinase inhibitor blocking tumor cell proliferation and angiogenesis whereas a second group of rats (n=6) was administered with Entinostat –MS-275- a benzoamide with activity against HDAC of class 1 and 2. The choice of these two compounds was supported by evidences in the literature reporting a significant reduction in the tumor mass size after 3/4 weeks of treatment.

The efficacy of the different drug administrations was monitored over approximately a 4-weeks time frame by running T₂-weighted anatomical MRI acquisitions. Results were then compared with those obtained in a control group (n=3) whose only received the drug vehicle.
Materials and Methods

Tumor Induction

One million of McA-RH7777 cells were collected and washed two times with PBS. Cells were then resuspended in 0.2 mL of DMEM medium and injected with a 27G needle under the hepatic capsula of the liver left lobe of anesthetized Buffalo rats.

Drug Administration and Animals Treatment

Sorafenib were dissolved in Cremophor EL and 95% ethanol (50:50) and administered at the dose of 30mg/kg in 500 μL vehicle once daily. Treatments started 1 weeks after cell inoculation and continued up to 24 days.

MS-275 will be suspended in methanol. This stock solution will be diluted at least 20-fold with water for injection to a final maximum concentration of 0.5% methanol before injection. Administration will be performed at the dose of 3mg/kg i.p. per day. Treatments started 1 week after cell inoculation and continued up to 24 days.

MRI Experiments

For MR experiments, animals were anaesthetized with isofluorane gas (about 1%) in a mixture of 33% O₂ and 66% N₂O. During the experiments, anaesthesia was maintained by adjustment of gas level as a function of breathing rate.

Transverse RARE T₂-weighted images were acquired using the following parameters:

- FOV=5.7X4.5cm;
- matrix size=156X156 (in-plane resolution of 0.365X0.288 mm²);
- slice thickness=2.5mm;
- number of slices=10;
- TR=1200ms;
- TE=53.3ms;
- RARE factor=4 and NEX=8.

For coronal images the slice thickness was set to 4mm, number of slices=7 and NEX=4.

These sets of parameters were chosen as a result of an optimization procedure aimed at achieving high resolution images with good contrast.

Tumor volume was measured through manual segmentation of the mass using default ParaVision ROI selection tools.

At the end of time of experiments, animals were sacrificed by an overdose of gas anesthesia and the livers excised for macroscopic evaluation.
Figure 2. Coronal and transverse T₂-weighted MR images at 0-3 days and 18-24 days intervals in: (A) untreated HCC rats; (B) MS-275 treated animals and (C) Sorafenib group. Red arrows indicate tumor masses.

Results
Both Sorafenib and MS-275 were effective in reducing tumor growth compared to animals treated with vehicle (see figure 1 and 2).

A significant reduction in the tumor growth rate has been registered in treated animals since the end of the 1st week of administration. This effect steadily increases over time reaching its maximum at the end of the study.

According to our results, Sorafenib exhibited the highest activity against hepatoma proliferation with an average tumor volume of 3174±142 mm³ detected after 24 days of treatment. Average values for MS-275 and controls animals were 8908±10.5mm³ and 9485±385mm³ respectively.

In addition, Sorafenib was also effective in limiting lung metastases and tumor spread to kidneys, spleen and peritoneum that affected 80-90% of the control and MS-275 animals as confirmed by our autoptic examination.

Conclusions
New Bruker Icon desktop MRI scanner proved itself as a valuable pre-clinical tool for the therapeutic follow-up of HCC rats undergoing 2 different drug treatments.

Operating at 1 Tesla Icon combined the advantages of reduced running cost and higher tissue contrast returning images with good resolution that allowed a full characterisation of our tumor models.

According to our results, drug treatment showed indeed a beneficial effect on cancer growth compared to controls. The highest reduction in tumor mass size was registered in rats after 24 days of treatment with Sorafenib (i.e. –66.54% in volume) compared to controls.
References
