

Predicting and Monitoring Response to Chemotherapy by Benzamide Riboside in Hepatocellular Carcinoma Using Apparent Diffusion Coefficient of Water

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Introduction

Hepatocellular carcinoma (HCC) and liver metastases from colon and breast cancers are an increasing problem worldwide, representing the third largest cause of cancer-related death (1). A non-invasive and accurate method for prediction and quantification of early chemotherapy response in intrahepatic HCC is highly desirable. Effective anticancer therapies damage and kill tumor cells, causing an increase in interstitial space due to cell shrinkage (in apoptosis) or rupture (in necrosis). Damage to tissue microvasculature may also lead to vasogenic edema, increasing the volume of extracellular space (ECS). These compartmental changes can be estimated using the apparent diffusion coefficient (ADC) of water measurement by diffusion-weighted (DW) ¹H MRI, as this method reflects mainly the changes in ECS. In this work, we studied the feasibility of water ADC measurement for predicting and monitoring response of HCC in the rat to chemotherapy by a new apoptotic agent, benzamide riboside (BR).

Methods

For the HCC model, one million N1S1 cells were inoculated in the left lateral lobe of the Sprague-Dawley (SD) rat liver (Fig. 1A). MR images were acquired with a Varian 9.4 T, 31 cm horizontal bore system. Each animal was examined weekly for 4 weeks after tumor cell inoculation. The effects of BR on tumor volume and water ADC were monitored using DW ¹H MRI. On day 15 after N1S1 cells inoculation, BR was infused through the hepatic artery using a microcatheter (Fig. 1B). The water ADC of the HCC and nearby liver tissue was measured with a 63 mm birdcage coil. A multi-slice DW ¹H imaging sequence with the following imaging parameters was used: 1,100 ms repetition time, 21 ms echo time, 256 × 128 data points over a 80 × 80 field of view, 0.5 mm slice thickness, 1.5 mm slice gap, and $b = 0, 256, 945, \text{ and } 1,679 \text{ s/mm}^2$. Respiratory gating was used to minimize the effects of motion on water ADC measurement. Total data collection time for a set of DW ¹H MRI at the four b values was 7.4 min.

Results

Two groups of BR-treated animals, which differed in their sensitivity to the treatment, were identified as responsive (R_{BR}) and non-responsive (NR_{BR}) (Fig. 2). In the R_{BR} group, tumor growth was arrested after therapy and the mean tumor volume in the R_{BR} group was $1/6^{\text{th}}$ and $1/16^{\text{th}}$ compared to the NR_{BR} group ($P < 0.05$) on days 7 and 14 after treatment, respectively (Fig. 3A). In the control and NR_{BR} groups, the tumors continued to grow throughout the study. Before BR treatment, the mean water ADC in tumor was higher in the R_{BR} group ($1.7 \times 10^{-3} \text{ mm}^2/\text{s}$) compared to the NR_{BR} group ($1.1 \times 10^{-3} \text{ mm}^2/\text{s}$, $P < 0.05$) (Fig. 3B). ADC in the control group was $1.3\text{--}1.4 \times 10^{-3} \text{ mm}^2/\text{s}$. In all three groups, the mean water ADC in the normal liver tissue was almost identical and unchanged throughout the study. The mean water ADC in HCC of all three groups was significantly higher compared to the adjacent normal liver. Tumor ADC and HCC-to-liver ADC ratio did not change significantly with tumor growth in control and NR_{BR} groups or with arrest of tumor growth in R_{BR} group.

Discussion

Water ADC of HCC is higher than in nearby normal liver tissue reflecting less packing of tumor cells and increase in ECS. Our data demonstrate that before BR treatment, the mean water ADC was higher in the R_{BR} group compared to the NR_{BR} group. It is unclear how the initial ADC values can predict tumor chemosensitivity, which could be clinically relevant. The tumors with high initial water ADC may be more vulnerable to BR therapy because of a weaker physiological condition, due to an increase in cell membrane permeability. These data are in agreement with an earlier report which showed that subcutaneously-implanted 9L gliosarcomas that respond to 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) treatment have higher pretreatment water ADC and ²³Na MRI signal intensity compared to control and non-responsive tumors (2). Both water ADC and tissue sodium content have been suggested to reflect changes in ECS (3).

It has been reported that the post-treatment increase in necrosis and/or apoptosis leads to an increase of water ADC in tumor due to an increase in ECS (3). However, that was not seen in our study. After BR infusion, the water ADC remained unchanged in both R_{BR} and NR_{BR} groups. In our experiments, it seems that BR-induced apoptotic and necrotic changes that may lead to increases in ECS and hence water ADC are counterbalanced by other factors that reduce ADC. Histological analysis has shown that HCC contains a conjugative type of necrosis, characterized by well-packed nucleus-less cells that can restrict water motion, and thereby increase the tortuosity of this space leading to restriction of water diffusion (4). Another factor that can reduce ADC may be the changes in water diffusion in the intracellular space (ICS). Usually, this component of tissue water ADC is underestimated even though cells occupy 82-85 percent of the tissue space in the liver (5). The experimental measurements of ADC in ECS and ICS showed that ADC values in these compartments are identical (6). The slight decrease in water ADC in the control group can be explained by a decrease in bioenergetic status of tumor tissue, due to development of hypoxic conditions and the resulting decrease in energy-dependent cytoplasmic motion.

Conclusion

Intrahepatic infusion of BR is a semi-effective treatment of HCC in rats. Pre-treatment water ADC value can predict the efficiency of the BR therapy. A higher initial ADC level could be a promising sign for effective BR treatment and, in contrast, tumors with a lower initial ADC value are more likely to be resistant to BR treatment. In certain cases water ADC may not change even with effective treatment because it is influenced by a number of compartmental, physiological, and metabolic changes which may counterbalance each other.

References: 1) Llovet JM, Beaugrand M. *J Hepatol* 2003; 38 Suppl 1:S136-149. 2) Babsky A et al. *J Magn Reson Imaging* 2006; 24(1):132-139. 3) Koh DM, Collins DJ. *Am J Roentgenol* 2007; 188(6):1622-1635. 4) Pfeuffer J et al. *Magn Reson Imaging* 1998; 16:1023-1032. 5) Makos JD et al. *J Appl Physiol* 1998; 85(5):1800-1805. 6) Duong TQ et al. *Magn Reson Med* 1998; 40(1):1-13.

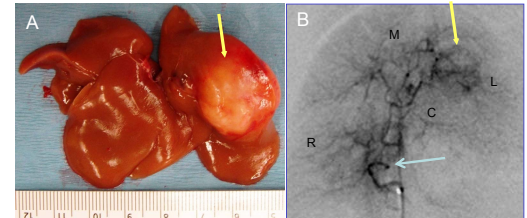


Fig. 1. HCC (28 days after N1S1 cells inoculation, A) and hepatic angiography via transfemoral approach (B). Yellow arrows show untreated HCC location and blue arrow shows the tip of Excel-14 microcatheter. R, M, C, and L – right, medium, central, and left lobes of the liver, respectively.

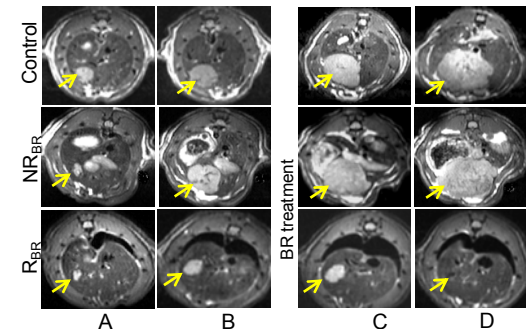


Fig. 2. Representative T_2 -weighted MRI of HCC in control, NR_{BR} , and R_{BR} groups on days 7 (A), 14 (B), 21 (C), and 28 (D) after N1S1 cells inoculation. Arrows point to HCC.

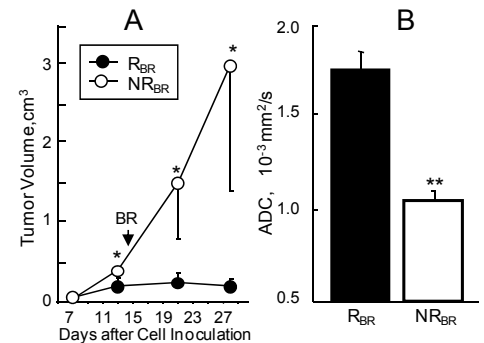


Fig. 3. Tumor volume changes (A) and water ADC (7 days before treatment, B) in R_{BR} and NR_{BR} intrahepatic HCC (n = 5). Significance: * - $P < 0.05$ (Tumor volume: vs. Day 7), ** - $P < 0.05$ (ADC: R_{BR} vs. NR_{BR}).