Variability of Apoptosis and Response in N1-S1 Rodent Hepatomas to Benzamide Riboside and Correlation to Early Changes in Water Apparent Diffusion Coefficient and Sodium MR Imaging

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ABSTRACT

Purpose: This pilot trial assesses variability of apoptosis and response 1 day after hepatic intraarterial (IA) benzamide riboside (BR) in rodent hepatomas and its correlation to water apparent diffusion coefficient (ADC) and single-quantum (SQ) and triple-quantum–filtered (TQF) sodium-23 (^{23}Na) magnetic resonance (MR) imaging.

Materials and Methods: Sprague–Dawley rats (n = 8) were inoculated with 10^6 N1-S1 cells. IA BR (20 mg/kg) was infused after 14 days. Animals were killed 1 day (n = 4) or 21 days (n = 4) after therapy. Imaging was performed 1 day before and after treatment. Volume was assessed over 2 weeks. Percentage apoptosis was counted from terminal deoxynucleotidyl transferase dUTP nick-end labeling–stained slides at 400× magnification. Kruskal–Wallis tests were used to compare apoptosis, and Wilcoxon signed-rank tests were used to compare MR signal intensity (SI).

Results: Apoptosis was marginally greater in tumor than in nontumor (6.7% vs 1.3%; $P = .08$), varying from 2% to 10%. Before treatment, MR SI was greater in tumor than in nontumor (ADC, 1.18 vs 0.76 [$P = .0078$]; SQ, 1.20 vs 1.04 [$P = .03$]; TQF, 0.55 vs 0.34 [$P = .03$]). After treatment, tumors increased in volume (0.62 vs 0.33; $P = .016$) variably over 2 weeks. MR SI remained greater in tumor than in nontumor (ADC, 1.20 vs 0.77 [$P = .0078$]; SQ, 1.76 vs 1.15 [$P = .016$]; TQF, 0.84 vs 0.49 [$P = .03$]). SQ and TQF SI increased by 47% ($P = .016$) and 53% ($P = .016$) in tumors, whereas ADC did not change.

Conclusions: Apoptosis was marginal and varied from 2% to 10%. Water ADC, SQ, and TQF MR imaging distinguished tumor from nontumor. Changes in water ADC and sodium MR imaging correlated to apoptosis and volume in select cases, but additional animals are needed to validate this trend against tumor growth.

ABBREVIATIONS

ADC = apparent diffusion coefficient, BR = benzamide riboside, HCC = hepatocellular carcinoma, IA = intraarterial, IMPDH = inosine monophosphate dehydrogenase, NAD = nicotinamide adenine dinucleotide, SI = signal intensity, SQ = single-quantum, TQF = triple-quantum–filtered, TUNEL = terminal deoxynucleotidyl transferase dUTP nick-end labeling

Benzamide riboside (BR) is an antimetabolite that is cytotoxic to multiple cancer cell lines (1–5). Tumor cells metabolize BR intracellularly, forming an analogue of nicotinamide adenine dinucleotide (NAD) that results in inhibition of inosine monophosphate dehydrogenase (IMPDH) and malate dehydrogenase. This depletes guanylates necessary

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for DNA replication and RNA synthesis. A rodent model has been developed for imaging and selection of the left hepatic artery to evaluate intraarterial (IA) drug administration for treatment of liver cancers (6). Studies that used VX2 rabbit and N1-S1 rodent hepatomas illustrated IA BR infusion to be effective at inducing apoptosis and tumor shrinkage at doses as high as 20 mg/kg (7,8). The only toxicity reported was a reversible hind-limb paralysis in rabbits at a dose of 20 mg/kg provided intraperitoneally every 48 hours for 2 weeks, which was thought to be a result of NAD depletion.

Noninvasive measurements of water apparent diffusion coefficient (ADC) by proton diffusion-weighted magnetic resonance (MR) imaging have shown potential to characterize pathologic changes in liver (9–11). Hepatocellular carcinoma (HCC) may be clinically differentiated from normal liver parenchyma, even though variations may occur with necrosis, diffusive path tortuosity, and integrity of cell membranes (12,13). In response to therapy, early cellular swelling and subsequent apoptosis may be visualized as an immediate decrease and later increase in water ADC, respectively (14). However, water ADC measurements may be biased as a result of respiratory, cardiac, and vascular perfusion motion artifacts (14).

Changes in the total tissue and intracellular sodium ions monitored by single-quantum (SQ) sodium-23 (^{23}Na) MR imaging and triple-quantum–filtered (TQF) ^{23}Na MR imaging, respectively, may also reflect physiologic, structural, and/or metabolic adaptations of tumor cells upon therapy (15,16). Sodium MR imaging is additionally insensitive to motion artifact, making it a promising adjunct for imaging tumor response in vivo.

Previous evaluations correlating water ADC and ^{23}Na MR signal intensity (SI) demonstrated SQ and TQF ^{23}Na MR SI to be greater in untreated HCC than normal parenchyma, whereas water ADC did not change during tumor growth (17). Early changes in water ADC and ^{23}Na MR imaging after nitrosourea infusion in subcutaneous 9L gliosarcomas were also reported as sensitive biomarkers for tumor shrinkage (18). The use of ^{23}Na MR imaging has been endorsed by various similar tumor studies (19–21). Histologic evidence to validate mechanisms of elevated MR SI is still essential.

We hypothesize that tumor response and apoptosis induced 1 day after IA delivery of BR will correlate to early elevations in water ADC and SQ and TQF ^{23}Na MR SI. The purpose of this study was to introduce a new series of experiments to test this hypothesis via a small pilot trial (i) to assess the variability of apoptosis induced 1 day after IA BR administration and (ii) to determine how variations in water ADC and ^{23}Na MRI SI 1 day after therapy relate to tumor response and apoptosis. Endpoints for response were defined as (i) tumor volume measured by ^{1}H MR imaging 24 hours before and after treatment in a first group of animals (group 1) and over a period of 2 weeks in a second group (group 2), (ii) tumor cell apoptosis measured 24 hours after treatment of animals from group 1, (iii) water ADC as measured by diffusion-weighted ^{1}H MR imaging, and (iv) SQ and TQF ^{23}Na MR SI measured 24 hours before and after treatment in animals from both groups. Apoptosis, water ADC, and SQ and TQF ^{23}Na MR SI in normal liver sections surrounding the tumor were assessed as secondary endpoints to evaluate drug toxicity to normal liver and to serve as an internal control for respective correlations to tumor MR SI.

MATERIALS AND METHODS

BR was synthesized as previously described (22). Animal studies were approved by the institutional animal care and use committee and are in compliance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. In preparation for imaging, animals were anesthetized with 1%–1.5% isoflurane provided by mask to effect. Animals were monitored for depth of anesthesia during the procedure and were allowed to recover following protocol termination. After surgery, buprenorphine (0.01–0.05 mg/kg) was administered subcutaneously every 8–12 h as needed for pain. Euthanasia was performed by lethal injection of barbiturate. Death was assured by exsanguination and removal of vital organs.

A total of eight Sprague–Dawley rats weighing 540–580 g were inoculated with 1×10^{-6} N1-S1 cells suspended in 0.05 mL into the left lobe of the liver by using a tuberculin syringe and a 30-gauge needle. Four tumors were allowed to grow for 15 days (group 1), whereas four were grown for 5 weeks (group 2). MR imaging of the liver was performed in vivo with a 9.4-T, 31-cm horizontal-bore scanner (Varian, Palo Alto, California). Diffusion-weighted ^{1}H MR imaging was performed by using birdcage volume coils tuned to 400 Hz. Sodium MR imaging was performed by switching to a loop-gap volume resonator tuned to 100 Hz with gradient-echo imaging sequence. Detailed discussion of imaging protocol and analysis used for MR imaging is provided in the protocol of another study that monitored growth in N1-S1 hepatomas (17).

Two weeks after tumor inoculation, eight rats were treated with 20 mg/kg BR by IA infusion into the left hepatic artery by the transfemoral approach as described previously (6). Tumor volumes were measured at 1 week after inoculation (ie, day −7), 24 hours before treatment (ie, day −1), and 24 hours after treatment (ie, day 1) on the basis of fat-suppressed T2-weighted images obtained by ^{1}H MR imaging. Tumors grown beyond 24 hours after treatment ($n = 4$) were also measured for volume at 1 week (day 7) and 2 weeks after treatment (day 14). Diffusion-weighted MR imaging was used on all animals to measure water ADC, and SQ ^{23}Na MR imaging and TQF ^{23}Na MR imaging were used on animals to determine SI relative to an NaCl reference signal 24 hours before (day −1) and after (day 1) treatment. Twenty-four hours after treatment, the four animals from group 1 were euthanized and the livers