LABORATORY INVESTIGATION

Relative Initial Weight Is Associated with Improved Survival without Altering Tumor Latency in a Translational Rat Model of Diethylnitrosamine-Induced Hepatocellular Carcinoma and Transarterial Embolization

Ryan M. Kiefer, BS, Stephen J. Hunt, MD, PhD, Santiago Pulido, BS, Stephen Pickup, PhD, Emma E. Furth, MD, Michael C. Soulen, MD, Gregory J. Nadolski, MD, and Terence P. Gade, MD, PhD

ABSTRACT

Purpose: To test the hypotheses that (*i*) heavier rats demonstrate improved survival with diminished fibrosis in a diethylnitrosamine (DEN)-induced model of hepatocellular carcinoma (HCC) and (*ii*) transarterial embolization via femoral artery access decreases procedure times versus carotid access.

Materials and Methods: One hundred thirty-eight male Wistar rats ingested 0.01% DEN in water ad libitum for 12 weeks. T2-weighted magnetic resonance imaging was used for tumor surveillance. Rats underwent selective embolization of $\geq$ 5 mm tumors via carotid or femoral artery catheterization under fluoroscopic guidance. Rats were retrospectively categorized into 3 groups by initial weight ($<$ 300, 300–400, $>$ 400 g) for analyses of survival, tumor latency, and fibrosis. Access site was compared relative to procedural success, mortality, and time.

Results: No significant differences in tumor latency were related to weight group ($P = .310$). Rats weighing $<$ 300 g had shorter survival than both heavier groups (mean, 88 vs 108 d; $P < .0001$), and more severe fibrosis ($<$ 300 g median, 4.0; 300–400 g median, 1.5; $>$ 400 g median, 1.0; $P = .015$). No significant difference was found in periprocedural mortality based on access site; however, procedure times were shorter via femoral approach (mean, 71 $\pm$ 23 vs 127 $\pm$ 24 min; $P < .0001$).

Conclusions: Greater initial body weight resulted in improved survival without prolonged tumor latency for rats with DEN-induced HCCs and was associated with less severe fibrosis. A femoral approach for embolization resulted in decreased procedure time. These modifications provide a translational animal model of HCC and transarterial embolization that may be suited for short-term survival studies.

ABBREVIATIONS

CCA = common carotid artery, CFA = common femoral artery, DEN = diethylnitrosamine, HCC = hepatocellular carcinoma

Given that a minority of patients with hepatocellular carcinoma (HCC) present with resectable disease, locoregional therapies such as transarterial (chemo)embolization provide important means of tumor suppression and can improve survival (1–3). Although long-term survival remains low as a result of recurrence and de novo tumor growth, developments in cancer biology hold promise for increasing the effectiveness of endovascular techniques through

From the Penn Image-Guided Interventions Laboratory (R.M.K., S.J.H., S.Pu., G.J.N., T.P.G.) and Departments of Radiology (S.J.H., S.Pi., M.C.S., G.J.N., T.P.G.) and Pathology and Laboratory Medicine (E.E.F.), Perelman School of Medicine at the University of Pennsylvania, 3400 Spruce St., Philadelphia, PA 19104. Received December 13, 2016; final revision received March 28, 2017; accepted March 29, 2017. **Address correspondence to** T.P.G.; E-mail: terence.gade@uphs.upenn.edu

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Appendix A and Figure E1 are available online at www.jvir.org.

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molecular and cell-based therapies (4–6). To advance these therapies toward clinical trials, translational animal models that recapitulate the complexity of human disease and mitigate the challenges of endovascular interventions must be developed.

Currently, a number of animal models of HCC exist, and each has been purported to offer unique benefits. Small animal models typically carry lower costs than large animal models, but, until recently, had been thought too small to allow for selective endovascular treatments (7). Xenograft models of HCC allow for reproducible tumors that are easily detected with short latencies, but fail to accurately recreate the tumor microenvironment of underlying liver injury that characterizes human disease (8–11). Similarly, orthotopic tumor models lack the phenotypic background of cirrhosis as well as the arterial dependence of HCC that characterizes human pathogenesis. These features can be reproduced by using autochthonous models such as the chemically induced diethylnitrosamine (DEN) model (8,12,13).

Segmental transarterial embolization in a DEN-induced rat model of HCC has previously been described (7). Two limitations we sought to address from that model were the duration and complexity of the procedure and the short survival window after tumor induction. Previous reports have described embolization via laparotomy or carotid arterial access (7,10,14,15). Femoral access has been successfully demonstrated as a feasible approach for hepatic angiography in a rat, but embolization outcomes have not been reported (16,17). Dose-dependent liver injury has been demonstrated in the DEN-induced rat model with controlled drug delivery via gastric gavage; however, this method is resource-intensive (18,19). Previous research has found that ad libitum mammalian water consumption is inversely related to body weight (20,21). As such, animals with greater body weight would be predicted to consume less toxin relative to body weight and theoretically develop less severe cirrhosis. In the present study of a DEN-induced rat model of HCC, we hypothesized that rats with larger initial weight would have lower cirrhotic disease burden and increased survival, and that a femoral artery approach to embolization would produce more favorable treatment parameters compared with a carotid artery approach.

MATERIALS AND METHODS

Complete experimental details are provided in **Appendix A** (available online at *www.jvir.org*).

Animal Model

Institutional animal care and use committee approval was granted before the start of the experiment. One hundred thirty-eight male Wistar rats (Charles River Laboratories, Wilmington, Massachusetts) were acclimated to the animal facility for 2 weeks before administration of ad libitum 0.01% DEN (Sigma-Aldrich, St. Louis, Missouri) in water for 12 weeks.

Rats were retrospectively assigned to weight cohorts based on their body weight at the initiation of the diet. The cutoff value for weight cohorts was determined to ensure that each cohort demonstrated commensurate growth based on published growth curves to limit confounding (22). The < 300 g group (approximate age, 5–7 wk) consisted of 70 rats weighing an average of 151 g (range, 115–183 g) at the start of the DEN diet. Of note, data from a portion of the < 300 g group have been previously analyzed and presented separately from the analyses provided in the present study (7). The 300–400 g group (approximate age, 7–11 wk) consisted of 23 rats weighing an average of 354 g (range, 301–392 g) at the start of the diet. The > 400 g group (approximate age, 9–15 wk) included 45 rats weighing an average of 442 g (range, 403–490 g) at the start of the diet. All data from the 300–400 g and > 400 g groups are unique to the present study. Sixteen rats in the < 300 g group died before completing the diet, and two rats from the > 400 g group were removed from the diet as a result of lethargy and were later restarted, but were not included in statistical analyses of tumor latency or survival as a result of the interruption in treatment. Eleven rats had inexact dates of death and were excluded. A total of 125 rats with exact documented dates of death or procedure were used for survival analysis. For analyses of cohort weight gain, rat weights were averaged within weight groups for each day of the diet. A cohort of 26 rats housed individually or together with another rat was used to analyze the influence of cohabitation on ad libitum DEN water intake. To record DEN intake, water bottles were weighed to track consumption throughout the diet. For rats under paired housing conditions, the total water consumption was averaged between the two rats.

Magnetic Resonance Imaging

Within the first 3 days of completing the DEN diet, rats underwent initial T2-weighted magnetic resonance (MR) imaging to monitor for tumor initiation and growth. Thereafter, weekly MR imaging was performed until rats were found to have at least one tumor measuring ≥ 5 mm as described previously (7).

Transarterial Embolization

After the development of radiographically evident ≥ 5-mm HCC tumors, 34 rats were scheduled to undergo embolization. Some rats in which HCC developed died before they could undergo embolization, and others were allocated for use in a parallel study (**Table 1**). Embolization of HCC tumors was performed under fluoroscopic guidance with the use of an AngioStar Plus Imaging System (Siemens, Malvern, Pennsylvania) via a left common carotid artery (CCA) or right common femoral artery (CFA) approach. The first 13 rats treated were all from the < 300 g group and were embolized via a carotid approach, and the later cohort consisted of 21 rats in the 300–400 g and > 400 g groups treated via femoral approach. Procedures were performed under inhaled anesthesia with 2% isoflurane