LABORATORY INVESTIGATION

Segmental Transarterial Embolization in a Translational Rat Model of Hepatocellular Carcinoma

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ABSTRACT

Purpose: To develop a clinically relevant, minimally invasive technique for transarterial embolization in a translational rat model of hepatocellular carcinoma (HCC).

Materials and Methods: Oral diethylnitrosamine was administered to 53 male Wistar rats ad libitum for 12 weeks. Tumor induction was monitored using magnetic resonance imaging. Minimally invasive lobar or segmental transarterial embolization was performed through a left common carotid artery approach. Necropsy was performed to evaluate periprocedural mortality. Histologic analysis of tumors that received embolization was performed to assess percent tumor necrosis.

Results: Severe cirrhosis and autochthonous HCCs were characterized in a cohort of rats composed of two groups of rats identically treated with diethylnitrosamine with median survival times of 101 days and 105 days (n = 10/group). A second cohort was used to develop minimally invasive transarterial embolization of HCCs (n = 10). In a third cohort, lobar embolization was successfully performed in 9 of 10 rats and demonstrated a high rate of periprocedural mortality (n = 5). Necropsy performed for periprocedural mortality after lobar embolization demonstrated extensive tissue necrosis within the liver (n = 3) and lungs (n = 2), indicating nontarget embolization as the likely cause of mortality. In a fourth cohort of rats, a segmental embolization technique was successfully applied in 10 of 13 rats. Segmental embolization resulted in a reduction in periprocedural mortality ($P = .06$) relative to selective embolization and a 19% increase in average tumor necrosis ($P = .04$).

Conclusions: Minimally invasive, segmental embolization mimicking the currently applied clinical approach is feasible in a translational rat model of HCC and offers the critical advantage of reduced nontarget embolization relative to lobar embolization.

ABBREVIATIONS

CHA = common hepatic artery, DEN = diethylnitrosamine, H&E = hematoxylin-eosin, HCC = hepatocellular carcinoma, LCCA = left common carotid artery

Advances in tumor biology have led to the identification of molecular and cell-based therapies that may augment the therapeutic effect of transarterial embolization or transarterial chemoembolization, or both (1,2). Translation of these therapies requires an animal model that recapitulates the biology of the human disease, while allowing the use of a minimally invasive embolization technique that reflects current clinical protocols. Animal models of transarterial chemoembolization reported in the literature underscore the challenge of developing a model with these attributes.

Although the ideal animal model would encompass the varied and complex biology embodied by hepatocellular carcinoma (HCC), two features are of particular

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relevance with respect to transarterial embolization and transarterial chemoembolization. First, most human HCCs develop in the background of cirrhosis, which has been shown to play a role in pathogenesis (3,4). The resultant hepatocellular dysfunction influences the approach to transarterial embolization or transarterial chemoembolization, or both, given the associated diminution in hepatic reserve and potential for vascular abnormalities. This dysfunction emphasizes the importance of superselective over lobar embolization to preserve functional hepatic parenchyma and to enhance therapeutic efficacy (5,6). Second, the dependence of HCCs on the arterial blood supply develops gradually through the growth of abnormal intranodular arteries, a feature that has been shown to occur in autochthonous tumor models and not in spontaneous tumor models (7,8). Chemically induced, autochthonous, small and large animal models of HCC encompassing both of these features have been described (9–11); however, these models have not been used for studies of transarterial embolization and transarterial chemoembolization preclinical application. Small animal models, including mice and rats, offer advantages including ease of handling, low procurement and maintenance costs, less extensive anesthesia and monitoring protocols during the procedure, and the potential for high throughput and established methods for biologic and genetic manipulation. The size of the vasculature in these animals has precluded the use of the clinically applied technique of minimally invasive, superselective embolization. For example, the diameter of the proper hepatic artery in Wistar rats weighing 250–300 g measures only 0.2–0.5 mm (12). None of the currently reported small animal models of transarterial embolization or transarterial chemoembolization, or both, describe selective embolization beyond the lobar hepatic artery.

In the present study, we describe and characterize the efficacy of minimally invasive, segmental transarterial embolization in a translational rat model of HCC. Cirrhosis and subsequent autochthonous HCCs were induced through the ad libitum oral administration of the hepatocarcinogen diethylnitrosamine (DEN). Embolization of either lobar or segmental hepatic arteries was achieved through a left common carotid artery (LCCA) approach with primary closure of the arteriotomy and sustained patency of the LCCA. This technique enables the opportunity for studies of transarterial embolization or transarterial chemoembolization, or both, in a clinically relevant model of HCC.

MATERIALS AND METHODS

Animal Model

Animal studies were conducted according to institutionally approved protocols for the safe and humane treatment of animals. Male Wistar rats 4 weeks old (Charles River Laboratories International, Inc, Wilmington, Massachusetts) were acclimated to the animal facility for 2 weeks. Autochthonous HCCs were induced in rats (n = 53) using ad libitum oral intake of 0.01% DEN (Sigma-Aldrich, St. Louis, Missouri) for 12 weeks, and the rats were divided among four cohorts (**Table**) (13). The water bottles were changed twice per week with freshly prepared 0.01% DEN. Animals were closely monitored over the course of therapy and weighed twice weekly. Single primary operator cohorts of 10 animals exposed to DEN (groups 1 and 2), baseline growth (eg, weight gain), and median survival were characterized for untreated (no embolization was performed) rats. Weight gain from these baseline groups was compared with published data for male Wistar rats not receiving DEN (14). Animals demonstrating weight loss > 20 g or diminished grooming capability were euthanized using inhaled carbon dioxide (20%). Necropsy was performed on euthanized animals; selected animals were submitted for formal necropsy performed by a veterinary pathologist.

MR Imaging

Magnetic resonance (MR) imaging was performed 1 day after the cessation of DEN treatment and weekly thereafter until the time of experiment or expiration. MR imaging was performed using a Varian 4.7-tesla 40-cm horizontal bore MR spectrometer with a 25 gauss/cm gradient tube interfaced to a Varian DirectDrive console (Agilent Technologies, Santa Clara, California). Animals were induced and maintained under inhalational anesthesia using isoflurane (2%) and positioned within a high-pass birdcage resonator that was tuned and matched to 200 MHz. Respiratory gated, T2-weighted images were acquired in the axial plane with 55 mm field of view, 128 × 128 matrix size, 430 μm in-plane resolution, 2 mm slice thickness, 0 interslice gap, 4 signal averages, 6.3 ms repetition time, and 2.98 ms echo time.

Table. Experimental Cohorts

Cohort	Animals per Cohort	Number of Groups
Tumor model characterization	10	2
Transarterial embolization development	10	1
Selective transarterial embolization	10	1
Superselective transarterial embolization	13	1

Note–There were 53 animals treated with diethylnitrosamine. In the first cohort, two groups of 10 animals each were used to characterize diethylnitrosamine-induced cirrhosis and carcinogenesis and overall survival without transarterial embolization (tumor model characterization). A second cohort of 10 animals was used to develop the applied method of transarterial embolization (transarterial embolization development). A third cohort of 10 animals underwent lobar embolization (lobar transarterial embolization). A fourth cohort of 13 animals underwent segmental embolization (segmental transarterial embolization).