N-Nitrosodiethylamine-Induced Pig Liver Hepatocellular Carcinoma Model: MRI with Gd-BOPTA enhancement

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Introduction Pigs are commonly used in surgical and interventional experiments because of their comparable size to human anatomy. In this study, liver HCC with a cirrhosis background was induced in three China Taihu pigs by N-nitrosodiethylamine, a potent chemical hepatocarcinogen [1,2], and the HCC nodules' MRI characteristics pre- and post- Gd-BOPTA-enhancement was studied. Gd-BOPTA distributes extracellularly in the first a few minutes after injection, then undergoes a duel route of elimination with approx 96% excreted renally and the remaining taken up by functioning hepatocytes and excreted in the bile [3,4]. The fraction taken up into the hepatocytes demonstrates high T1 relaxivity and is responsible for a marked and long-lasting enhancement of the normal liver parenchyma [5].

Materials and Methods

Starting at 2-month old, liver HCC was induced in three China Taihu pigs by intraperitoneal injection of 10 mg/kg N-nitrosodiethylamine (DENA, N-0756; Sigma) once a week for 3 months, followed by a period of 10–12 months without DENA treatment. Prior to MRI, X-ray CT was carried out to confirm the existence of HCC nodules in the liver of all three pigs. Axial MR images of the liver were obtained using a 1.5T MR imager (Magnetom Sonata, Siemens). Pre-contrast images were acquired with a TurboFLASH T1W sequence [resolution: 2.4*1.6mm, 8.0thk/2.4sp (mm)] and HASTE T2W sequence [resolution: 2.1*1.6mm 8.0thk/2.4sp (mm)]. Post-contrast images were acquired in the standard arterial, portal, equilibrium phases and 60 min after Gd-BOPTA 0.05mmol/kg (clinical dosage) administration with a T1W 2D Flash sequence [resolution: 2.4*1.6mm 8.0thk/2.4sp (mm)]. MR images were evaluated independently by three gastrointestinal radiologists. After MRI animals were euthanized. Portions of the liver tumor area were excised for micro-histology. Liver sections were prepared by producing 1-cm-thick slices from deep frozen specimens in the axial planes. These sections were photographed and compared with the corresponding MR images. All pigs were in generally good health until the end of the study.

Results

In total, 76 and 94 tumors nodules with diameter >/= 5mm were detected by pre-contrast (combined T1WI and T2WI evaluation) and post-contrast MRI (all phases images combined evaluation) in three pigs. On the 60min delayed MR images, the hepatic parenchyma demonstrated a marked enhancement. The ratio of hyper- vs hypo- vs heterogeneous- intensity nodule number was 0 vs 41 vs 15 on pre-contrast T1WI, and 45 vs 6 vs 9 on pre-contrast T2WI. The nodules were predominantly hypo-intensity on T1WI and hyper-intensity on T2WI. Some lesions showed iso-intensity hence undetectable. The ratio of hyper- vs hypo- vs heterogeneous intensity nodule number was 36 vs 14 vs 24 on the arterial phase images, 11 vs 23 vs 26 on the portal phase images, and 10 vs 23 vs 22 on equilibrium phase images, and 15 vs 31 vs 19 on 60min delayed images. It appeared that the lesions were predominantly hyper-intensity on arterial phase images, and predominantly hypo-intensity and iso-intensity on portal and equilibrium phase images. Hypo-intensity lesions dominated on 60min delayed images.

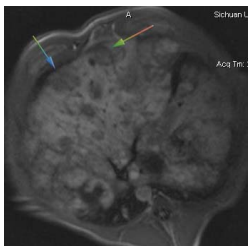

Fig1A

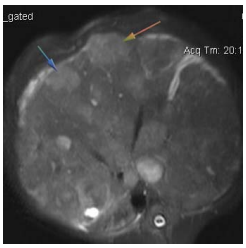

Fig1B

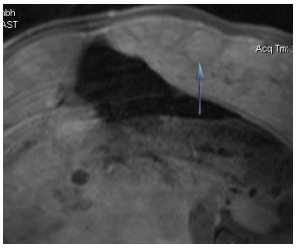

Fig2

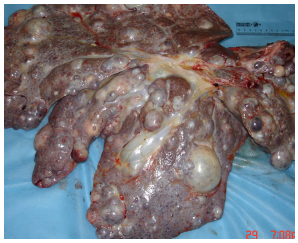

Fig3

At gross inspection, tumors occurred as multiple nodules and had a spheroid shape with dark-reddish, grayish, yellow-whitish, or gray-whitish colors. Sectional inspection was able to reveal not only those nodules detected by MRI but also many of the smaller nodules undetectable by MRI. By gross sectional inspection, there were 112 HCC nodules with diameter >/= 5mm in three pig's livers. The histological features of the induced tumor cells resembled human HCC in cirrhosis background. The tumor nodules within the same pig liver tended to display a varying degree of differentiation. According to the WHO criteria [6], all highly, moderately, and poorly differentiated HCCs were identified. Using the sectional inspection results as reference, the sensitivity of Gd-BOPTA enhanced MRI was significantly greater than those of pre-contrast images for all the three observers (80.4% vs 67.9%, 87.5% vs 70.5%, 83.0% vs 65.2% respectively; $p<0.05$), however, no significant difference was seen in the positive predictive value between Gd-BOPTA-enhanced and pre-contrast images for all the three observers (90.0% vs 86.4%, 89.9% vs 82.3%, 91.2% vs 84.9 respectively; $p>0.05$).

Fig1. Pre-contrast T1W image (A) and T2W image (B) show multiple DENA-induced HCC nodules in a pig liver which demonstrate varying MR signals. Two nodules with 'classic' T1WI hypo- and T2WI hyper- signal are indicated by arrows. Fig2. DENA-induced liver HCC nodules in the 60min delayed T1W MR image show the uptake of Gd-BOPTA into the lesions and demonstrate slight hyper-signal. Fig3. A pig liver specimen with DENA-induced HCCs shows multiple, multi-centric, encapsulated nodules.

Discussion This is the first study using Gd-BOPTA-enhanced MRI to assess DENA-induced HCCs in pig liver. The signal intensity on MR images could be an indicator of histological differentiation of HCC (7). The Gd-BOPTA dynamic enhancement characters follow the formula of blood supplying trait of HCC. The residual hepatocyte-like function of HCC cells can be assessed on the 60min delayed images. The MRI findings in this study agreed with histology results that all highly, moderately, and poorly differentiated HCC tumors were identified in the pig livers. Similar to human experience, in this study Gd-BOPTA significantly increased the sensitivity of MRI detection of HCC nodules. That HCC nodules of various histological differentiations with a variety of MRI signals and enhancement patterns co-exist in the same liver with comparable size to human anatomy provides a versatile animal model both for therapeutic investigation and diagnostic technology development, the latter includes MRI sequence optimization and also contrast agent research. It is noteworthy that increasing the dosage of DENA, addition of a promoter in the diet, such as Phenobarbital, or the administration of DENA at an earlier age may shorten the tumor induction time in this animal model.

Reference 1). Kinoshita A, et al. Carcinogenesis 2003; 24:1389. 2).Graw JJ, et al. Z Krebsforsch Klin Onkol; 1977 89:137. 3). Spinazzi A et al. Acad Radiol 1999; 6:282. 4). Spinazzi A et al. Acad Radiol. 1998; Suppl 1:S86. 5). Vogl TJ et al AJR 1992;158:887. 6). Gibson JB, Sobin LH. International histological classification of tumours. Geneva: WHO,1978:13. 7). Murakami T, et al. J Magn Reson Imaging. 1998;8:346-58.