Metabolic Imaging of Hepatocellular Carcinoma in HBsAg transgenic Mouse Model Treated With Aflatoxin B1: a New Biomarker

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Introduction: Aflatoxin-B1 (AFB1) and hepatitis-B virus (HBV) are the most common etiological factors associated with the high incidence of hepatocellular carcinoma (HCC) in Asia [1]. Epidemiological studies have also suggested a strong correlation between AFB1 exposure and a signature mutation on the tumor suppressor gene, p53. The latter is often mutated at codon 249 in HCCs that arise from areas of high risk factors, resulting in an amino acid change from arginine (AGG) to serine (AGT), which inactivates its function [2]. Thus we have investigated the HCC model that closely mimics human HCC, induced by expression of the Hepatitis surface antigen as a transgene from the albumin promoter (HBsAg) after treatment with AFB. In the present study we used magnetic resonance imaging (MRI) and spectroscopy (MRS) and in vitro MRS to expose the biochemical changes that are associated with a high risk of HCC.

Methods: All the *in-vivo* and *ex-vivo* experiments were done in compliance with and approved by the local Biological Resources Center (BRC). In this study we employed nine mice over three categories. The first group involved HBsAg mice treated with Aflatoxin B1 (AFB1) acronymed as HBsAg +AFB (n = 3). The second group (n = 3) consisted of HBsAg mice treated with corn oil (no AFB1 treatment). Third group included non-transgenic wild type mice (n = 3) injected with only corn oil, to serve as a control. AFB1 and corn oil treatment was performed on the 7th day after birth. The HBsAg mice develop HCCs around 9 months after AFB1 injection, which resemble the human HCC pathologically. All animals were subjected to localized MRS and diffusion tensor imaging (DTI). Prior to *in vivo* experiments, animals were anaesthetized with 1.5-2 % v/v isofluorane mixed with medical air and medical oxygen. All MR experiments were performed with a 7 T ClinScan MRI/MRS scanner (Bruker, Karlsruhe, Germany) equipped with a 72-mm volume resonator for RF transmission, in combination with a 20-mm surface receive-only coil. All experiments were respiratory gated to obviate the effects of liver motion. MRI experiments included gradient echo, spin echo; and multi-directional DTI (b = 0, 1000 s mm^{-2}) was employed with 20 gradient directions over the entire liver. A volume-localized PRESS sequence was employed on normal liver, and the tumor lesions, with TR = 4 s, TE = 13 ms, number of transients Av = 128, voxel size = 2 × 2 × 2 mm^3, spectral width = 3500 Hz, with 2048 complex points per free induction decay. The choline and carnitine concentrations were estimated from the MRS spectra using the unsuppressed water signal from the same location as an intensity standard. DTI data were processed using 'DTI studio' [3] to calculate the apparent diffusion coefficient (ADC). After completion of all *in vivo* experiments the intact tumor tissues were isolated and subjected to high-resolution magic angle spinning (HRMAS) and *in-vitro* NMR spectroscopy.

Results and Discussion: Figure 1 shows the *in vivo* ^{1}H NMR spectrum obtained from the HCC tumor of HBsAg + AFB1 mouse at the age of 54 weeks. Prominent resonances in the region of 0.78 ppm to 2.8 ppm were assigned to lipids. The resonance from the -N(CH$_3$)$_3$ of choline was detected at 3.20 ppm. In addition to the choline signal we also observed the carnitine peak at 3.38 ppm in tumors of HBsAg group treated with AFB1. The HRMAS spectrum (Figure 2) of intact tumor tissue, and spectra of perchloric acid extracts (not shown), confirmed the presence of carnitine via its resonance at 3.38 ppm. Additional 2D HRMAS spectra and *in vitro* studies were performed confirming the assignment of carnitine. The average choline-to-water ratio (Figure 3) in the HBsAg + AFB1 group of mice was 0.0039 ± 0.0002, compared to the control group at 0.0019 ± 0.0002 (p = 0.01). The average choline concentration in the wild-type group was 0.0015 ± 0.0001 (p = 0.005); while the average carnitine-to-water ratio in tumors of the HBsAg + AFB1 group was 0.00075 ± 0.0001. The carnitine resonance was absent from the control group of animals. Biosynthesis of carnitine involves the hydroxylation of trimethyllysine to 3-hydroxy-6-*N*-trimethyllysine. Then the hydroxytrimethyllysine is cleaved to yield butyrobetaine aldehyde that is then oxidized to butyrobetaine that in turn is finally hydroxylated to yield carnitine [4]. Carnitine plays a major role in fatty acid transport into the mitochondria, prior to β-oxidation and hence energy transduction. The average ADC value of the lipid in the tumors of HBsAg + AFB1 mice was 7.26 x 10^{-4} mm^2 s^{-1} compared to normal liver tissue where it was 9.67 x 10^{-4} mm^2 s^{-1} (p = 0.00001); this is consistent with the marked changes in cellularity.

Figure 1

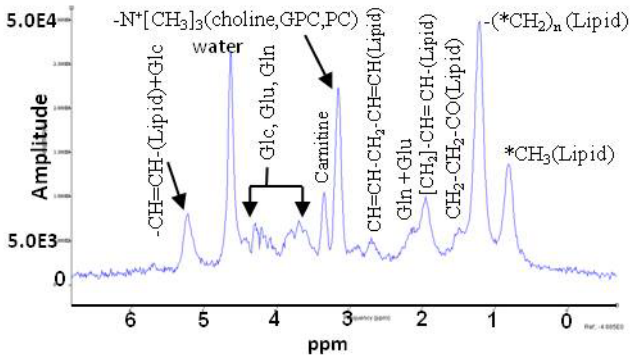
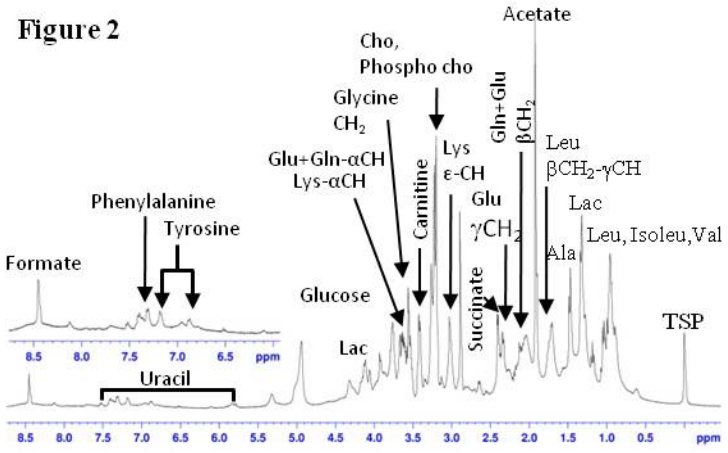


Figure 2



Conclusions: We investigated HCC *in vivo* in HBsAg transgenic mice model that simulated the human condition. We detected carnitine for the first time, to our knowledge, in HCC tumors of HBsAg + AFB mice model. Therefore relatively high concentrations of carnitine may be a biomarker for the early detection of HCC.

Figure 3

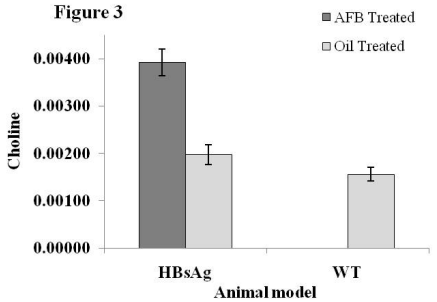
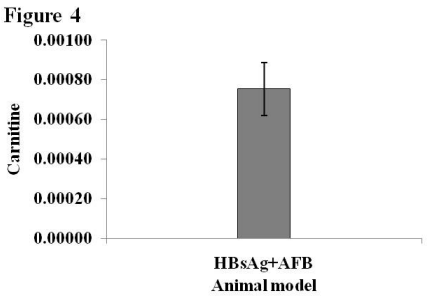


Figure 4



References: (1) Anthony PP. Hepatocellular carcinoma: an overview. Histopathology 2001;39:109–118. (2) Staib F, et al., TP53 and liver carcinogenesis. Hum Mutat. 2003; 21:201–216. (3) Susumo Mori et al., DTI, studio version 3.2.0. (4) Lindstedt G. et. al., J. Biol. Chem.1965;240:316-321.