Early Detection of Liver Tumors in Mice using MicroCT and a Hepatocyte-selective Contrast Agent

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Introduction

A major impediment in cancer research is the inability to accurately detect and monitor tumor growth in mouse models. Due to the recent advances in genetic manipulation, these models have become increasingly important. MicroCT scanners[1,2] capable of sub 50-micron spatial resolutions have recently been introduced that afford potential to more effectively monitor tumor growth and development in small animal models. Relatively long image acquisition times, however, preclude the use of conventional water-soluble contrast agents for tumor detection. The goal of this report is to evaluate ITG, a new hepatocyte-selective CT contrast agent that resides in the liver for several hours, for its ability to detect very small tumors in mice.

Targeting the Apo-E Receptor

Our strategy for the site-specific delivery of imaging agents to the liver is based on a biochemical approach, whereby naturally occurring compounds known to be stored or metabolized in the liver server as carriers for the radiologic moiety. Once absorbed by the intestinal tract, triglycerides are are incorporated into chylomicrons which are subsequently secreted into and transported through the thoracic duct until they reach the circulation. Following a rapid transfer of surface bound apoprotein C-II from circulating HDL to the chylomicrons (Fig 1) chylomicrons, now associated with apo C-II, are then acted upon by lipoprotein lipase in peripheral tissues including adipose, muscle and lung. After the natural triglyceride is removed from the core of the chylomicron the resulting triglyceride depleted chylomicron, know as a chylomicron remnant, rapidly acquires apo-E from the plasma. Once associated with apo-E, the small chylomicron remnants (<200 nm) enter the space of Disse and gain access to hepatocytes. Once associated with surface bound Apo-E receptors on hepatocytes, the remnants undergo endocytosis thought to be mediated by hepatic triglyceride lipase. This process is quite efficient in humans as the plasma half-life for clearance of chylomicrons and remnants following a meal is about 15 minutes. Humans are capable of processing up to 100 grams of triglyceride in this manner daily. Hepatocyte-targeted delivery based on the natural chylomicron-remnant/Apo-E receptor uptake pathway thus represents an attractive biomimetic mechanism for delivering lipophilic agents to the liver. Polyiodinated triglycerides were viewed as appropriate liver-specific carrier molecules for the design and synthesis of these hepatocyte-selective CT imaging agents.

Project Aim. The goal of this project was to evaluate the efficacy of a new hepatocyte-selective CT contrast agent, 1,3-bis [7-(3-amino-2,4,6-triiodophenyl) heptanoyl]-2-oleoyl-glycerol (ITG, Fig. 2) as a liver-selective, and microCT compatible, contrast agent for the early detection of liver tumors in both a xenograft and a transgenic mouse model.

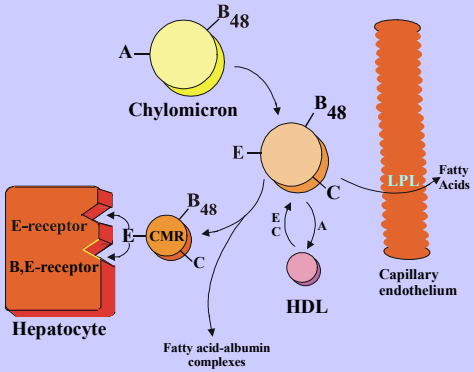
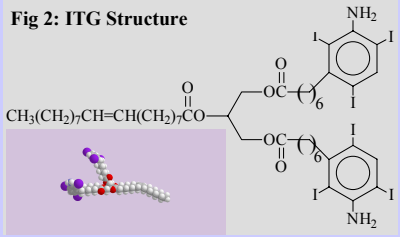


Fig 1. Chylomicron Remnant Delivery System

Fig 2: ITG Structure



Materials and Methods

ITG (Fig 2) was synthesized and formulated into a synthetic chylomicron remnant microemulsion according to our standard methods.[3,4] The final ITG preparation was tested consisting of 20% total lipid and administered as a single IV (tail vein) bolus dose (0.2 ml/20g bw) into Balb/C mice bearing CT-26 colon adenocarcinoma ($5x10^5$ cells/50µl) xenograft implants in the liver or alternatively into transgenic TGFα hepatoma bearing mice. Anesthetized mice were scanned 4 hours post injection using an ImTek microCT scanner (43 KVP, 410 µA, 390 steps) prior to and at three-day intervals for up to 21 days following tumor cell implantation in the xenograft model. Images were reconstructed (256x256x256 voxels/Shepp-Logan filter/no beam hardening correction/back projection over an appropriate sub volume) and subsequently displayed and analyzed using Amira 3-D visualization software (V2.3). Upon completion of final scanning, animals were sacrificed and radiologic-pathologic correlation performed.

Transgenic Mouse HCC Model. The development of spontaneous hepatocellular cancer in transgenic mice over expressing the TGFα gene has been extensively evaluated and is an extremely promising animal model for study of this disease.[5]

TGFα is a mitogen for epithelial cells and binds to the EGF receptor; unregulated expression of TGFα results in tumor formation. In male CD1 mice expressing the transgene TGFα under the control of the zinc-inducible metallothionine 1 (MT1) promoter, 75-80% develop HCC after 12 months of age. However, when the alkylating agent diethylnitrosamine (DEN), a chemical carcinogen, is used to induce tumor growth at 15 days of life, 90% of mice develop HCC by 6 months of age. On histologic examination, these tumors consist of well differentiated hepatocellular carcinomas of a solid pattern. Because the tumors arise spontaneously, we utilize these animals as a suitable model for preclinical studies.

Results and Discussion

Although image quality is excellent for high contrast structures like bone, differentiation of low contrast soft tissue structures is difficult with non contrast-enhanced microCT. Long-acting organ-selective contrast agents however, will improve detection of tumors in these organs significantly.

Liver uptake of ITG in mice followed a delayed pharmacodynamic pattern relative to other animal models examined. In mice, optimal liver enhancement occurs around 3-4 hours, whereas in other species including rat and dog optimal opacification occurs around 45-60 minutes following injection.

Utilizing ITG, a hepatocyte-selective contrast agent for microCT, it has been possible to consistently detect and accurately localize liver tumors less than 300 microns in diameter in both xenograft and spontaneous transgenic mouse models (Figs 3-5). Using this approach we have been able to accurately monitor tumor growth and it is also likely that it will be possible to monitor tumor regression in response to therapy. Moreover, utilizing this approach we have recently performed microCT- guided small needle biopsies of liver tumors in live mice thus enabling the potential to follow tumor histology serially over extended periods of time.

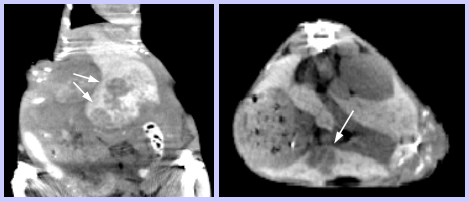


Fig 3. ITG-enhanced microCT image of CT-26 tumor implants in mouse liver 6 days post inoculation. Tumors (arrows) are clearly visible as low attenuation areas in both the coronal (left) and axial (right) views.

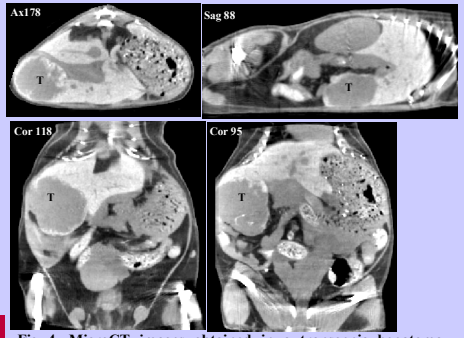


Fig 4. MicroCT images obtained in a transgenic hepatoma-bearing mouse 4 hours post ITG administration. Axial, sagital, and coronal views presented. Note viable hepatocytes adjacent to hepatoma cells in Cor 95 and 118. (Tumor = T).

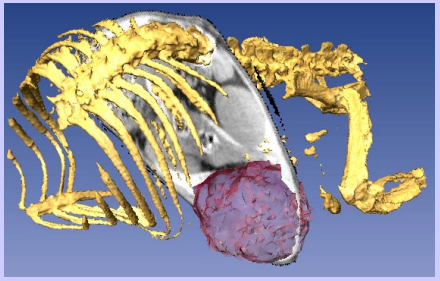


Fig 5. ITG-enhanced microCT 3D image of an anesthetized HCC-bearing TGFα mouse showing the segmentally rendered hepatoma within the axial slice and it relative location within the surface rendered skeleton.

Conclusions

This is the first time ITG or any CT contrast agent (to our knowledge) has been evaluated in live mouse liver tumor models. ITG, a hepatocyte-selective CT contrast agent afforded superior tumor edge detection capability. Due to its biomimetic nature, we feel that ITG offers the capability of providing not only superior anatomic capability, but also functional information capable of nearing or exceeding the spatial resolution of the CT scanners themselves.

References

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