MRI of Intrabiliary Delivery of Motexafin Gadolinium into Common Bile Duct Walls: In vitro and ex vivo evaluations

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PURPOSES: Motexafin gadolinium (MGd) is a multifunctional agent as an intracellular T1-weighted MR tumor selective contrast agent, an anti-tumor agent, and an biomarker emitting red fluorescence[1,2]. This study was to initiate the investigation of using MRI to monitor intrabiliary delivery of MGd into common bile duct (CBD) walls of pigs.

METHODS: The first phase study was designed to evaluate, in vitro, the capability of MGd entering human cholangiocarcinoma cells (Mz-ChA-1). Mz-ChA-1 cells, kindly provided by Dr. Gianfranco Alpini (Texas A&M Health Science Center), were cultured in Eagle's medium (DMEM) with different supplements for 24 hours. Then, these cells were treated by adding MGd at various concentrations of 25, 50, 75, 100, 125, and 150μg/mL for 48 hours. Cells were washed with phosphate-buffered saline (PBS), and then transferred to 0.6-mL Eppendorf tubes and dispersed in 200 μL of 1% agarose. In vitro 3T MRI of seven tubes for each of MGd-treated cell group were achieved with T1-weighted imaging (T1WI)(Turbo spin echo and TR/TE=550/12ms). Signal intensity (SI) of each tube was measured. Other groups of Mz-ChA-1 cells were treated by MGd at the same concentrations as described above, fixed with 4% paraformaldehyde, counterstained with DAPI, and then examined with a laser confocal microscope at 488-nm excitation and 700-nm emission.

The second phase study was designed to validate, ex vivo, the feasibility of intrabiliary local delivery of MGd into the CBD wall. Nine cadaveric livers with entire bile duct and portion of duodenum were harvested from pigs. Accessed via the Ampulla of Vater from the duodenum, a custom-made microporous balloon catheter was positioned into CBD, where 3-mL MGd (0.5mg/mL) was locally infused into the walls of five CBDs, while four CBDs were infused with 3-mL trypan blue mixed with 3% gadolinium or 3-mL PBS to serve as controls. Ex vivo 3T MRI of these CBDs were performed with fat-suppressed T1WI (Turbo spin echo and TR/TE: 550/12ms). The contrast-to-noise ratio (CNR) of CBD-to-tissue surrounding $CBD_{background}$ was calculated with the following equation: $CNR = (SI_{CBD} - SI_{background})/SD_{noise}$. Immediately after MR imaging, the CBD specimens were cryosectioned at 8-μm to detect MGd as yellow color spots and trypan blue as blue color spots under microscopy.

RESULTS:

Of the in vitro experiments, MGd presented its intracellular capability for Mz-ChA-1 cells, which was confirmed by (i) T1WI as a linear increase of SIs from 25 to 75-μg/mL MGd and a plateau pattern of SIs from 75 to 150-μg/mL MGd (Fig1); and (ii) confocal microscopy that demonstrated MGd as intracytoplasm pink dots of Mz-ChA-1 cells (Fig 2). Of the ex vivo experiments, T1WI demonstrated significant higher CNR in the MGd-infused CBD wall than that in the controlled CBD wall (144.5±17.3 vs 23±5.3, two-tailed Student *t*-test, $p<0.05$)(Fig.3). Histology confirmed MGd-staining as yellow-colored CBDs in comparison to trypan blue staining as blue- colored CBDs.

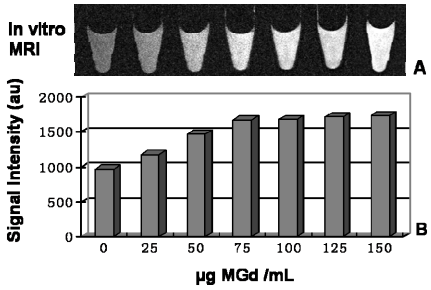

Fig.1. (A) In vitro T1WI of Mz-ChA-1 cells treated by MGd at different concentrations, showing increased bright signals as increase of MGd concentrations. (B) Measurement of signal intensities (SI) further confirms the findings of A, demonstrating a linear increase of SIs from 25 to 75-μg/mL MGd, followed by a plateau pattern of SIs from 75 to 150-μg/mL MGd.

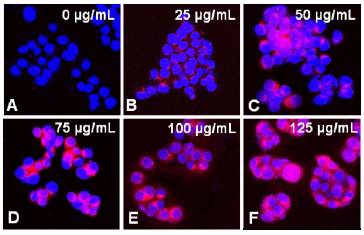

Fig.2. Confocal microscopy of Mz-ChA-1 cells treated by MGd at different-concentrations (B-F), showing increased intracellular uptakes of MGd, as pink dots, as the increase of MGd concentrations, which is not seen in the control cells (A).

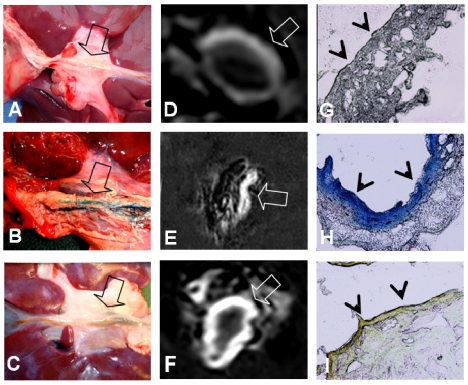

Fig. 3. (A-C) Ex vivo intrabiliary delivery of MGd, showing blue-colored CBD with trypan-blue/gadolinium infusion (B) and yellow-colored CBD with MGd infusion (C), which are not seen in control CBD with PBS infusion (A). Arrows indicate the CBD walls. (D-F) Ex vivo MRI, showing the greatest enhancement of the CBD wall with MGd infusion (F) than with trypan-blue/gadolinium infusion (E) and with PBS infusion (D). (G-I) Histology confirms the success delivery of MGd as yellow deposits (I) and trypan-blue as blue deposits in the CBD walls (arrowheads), which are not seen in the control CBD wall (G).

CONCLUSIONS:

This study initially confirms the intracellular property of MGd for human cholangiocarcinoma cells and demonstrates the potential of using MR imaging to monitor and guide the intrabiliary delivery of MGd into CBD walls. Continuous development of this technique may open up a new avenue for MR-guided interventional treatment of CBD strictures/obstructions resulted from different biliary diseases.

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References:
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