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Chimeric Mouse Model for MRI Contrast Agent Evaluation

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Abstract

Purpose: While rodents are the primary animal models for contrast agent evaluation, rodents can potentially misrepresent human organ clearance of newly developed contrast agents. For example, Gd-BOPTA has ~50% hepatic clearance in rodents, but ~5% in humans. This study demonstrates the benefit of chimeric mice expressing human hepatic OATPs (organic anion-transporting polypeptides) to improve evaluation of novel contrast agents for clinical use.

Methods: FVB (wild-type) and OATP1B1/1B3 knock-in mice were injected with hepatospecific MRI contrast agents (Gd-EOB-DTPA, Gd-BOPTA) and non-specific Gd-DTPA. T₁-weighted dynamic contrast enhanced MRI was performed on mice injected IV. Hepatic MRI signal enhancement was calculated per time point. Mass of gadolinium cleared per time point and percentage elimination via feces and urine were also measured.

Results: Following IV injection of Gd-BOPTA in chimeric OATP1B1/1B3 knock-in mice, hepatic MRI signal enhancement and elimination by liver was more reflective of human hepatic clearance than that measured in wild type mice. Gd-BOPTA hepatic MRI signal enhancement was reduced to 18% relative to wild-type mice. Gd-BOPTA elimination in wild type mice was 84% fecal compared to 32% fecal in chimeric mice. Hepatic MRI signal enhancement and elimination for Gd-EOB-DTPA and Gd-DTPA were similar between wild-type and chimeric cohorts.

Conclusion: Hepatic MRI signal enhancement and elimination of Gd-EOB-DTPA, Gd-BOPTA, and Gd-DTPA in chimeric OATP1B1/1B3 knock-in mice closely mimics that seen in humans. This study provides evidence that the chimeric knock-in mouse is a more useful screening tool for novel MRI contrast agents destined for clinical use as compared to the traditionally used wild-type models.

Keywords

OATP; MRI; chimera; pre-clinical; humanized model; gadolinium

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Introduction

Rodents have long been the primary initial *in vivo* models for the evaluation of new pharmaceuticals, including new contrast agents. Using rodents to screen new contrast agents for further research and development is convenient, with facile methods for imaging and measuring pharmacokinetics, but rodent organ systems do not fully mimic human systems and may in fact poorly predict how contrast agents will perform in humans. This is demonstrated by the *in vivo* pharmacokinetics of MRI contrast agents that are transported via organic anion-transporting polypeptides (OATPs) expressed by hepatocytes. For example, the FDA-approved contrast agent Gd-EOB-DTPA has ~50% hepatic clearance in both humans and rodents, but Gd-BOPTA has ~50% hepatic clearance in rodents and only ~5% in humans¹. Given such a drastic difference in organ clearance, using rodents with engineered biological systems that more closely mimic human physiology in early evaluation of new MRI contrast agents, and perhaps drugs in general, could increase success in clinical trials. The convenience of small animal imaging systems in combination with the translatability of chimeric biological systems in animals expressing human proteins would be optimal for such testing.

We hypothesize that since hepatic clearance of contrast agents is primarily mediated by hepatic OATPs, novel contrast agents could be evaluated more easily in rodents expressing human hepatic OATPs (OATP1B1 and OATP1B3) rather than mouse hepatic OATPs (OATP1A1). Human and murine OATPs are very similar proteins with 12 transmembrane helices, and are found in many types of tissue². OATP1B1 and OATP1B3 are expressed in the liver, OATP1A1 is expressed in the liver and kidney³. Both human and mouse hepatic OATPs transport bile acids, conjugated bilirubin, and hormones, along with exogenous molecules including statins, methotrexate, and some MRI contrast agents⁴. Additionally, expression of these transporters is determined by HNF1 α -dependent transcriptional regulation⁵. Pharmacokinetic differences between the human and mouse transporters may be explained by some structural difference, including the location of binding sites and intra- and extra-cellular loops^{3,6}.

A transgenic mouse expressing human hepatic OATPs instead of mouse hepatic OATPs, originally reported by Salphati et al.⁷, is currently commercially available from Taconic Biosciences. This chimera was engineered by first using Cre-mediated deletion to knock out the *Slco1A/1B* (OATP1A/1B) gene cluster in FVB mice, then inserting gene *Slco1B1* (OATP1B1) and gene *Slco1B3* (OATP1B3) under regulation of an Apolipoprotein E (ApoE) promoter, yielding liver-restricted expression. In this work, we sought to determine whether this mouse model would accurately recapitulate the organ clearance of two FDA-approved hepatospecific MRI contrast agents to that of human systems.

Methods

Materials

Male wild-type FVB mice purchased from Taconic were used for control mice. Chimeric OATP1B1/1B3 knock-in mice (Oatp1A/1B cluster knockout FVB background) were also purchased from Taconic Biosciences (Germantown, NY). Given such a large predicted effect

size in the difference of the hepatic uptake of contrast agents between wild-type mice and knock-in mice, we determined that cohorts of 3 mice per group would be sufficient. Data sets from a previous study using Oatp1A/1B cluster knockout mice from Taconic Biosciences were included in analysis⁸. Chimeric knock-in mice express OATP1B1/1B3 under the ApoE promoter, restricting expression to the liver. Clinical grade hepatospecific MRI agents Gd-EOB-DTPA (Eovist in USA and Primovist in Europe) and Gd-BOPTA (Multihance) and non-specific Gd-DTPA (Magnevist) were used. Rifampicin was from Sigma-Aldrich.

Imaging Experimental Design

Animal studies were approved by the Institutional Animal Care and Use Committee at Michigan State University and are in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80–23). Mice were anesthetized to effect with isoflurane, and tail veins were catheterized. Integrity of the catheter was tested with a 1-second flush, done by hand with a 1 ml syringe. For imaging, we used a 7T Bruker 70/30 BioSpec MRI system running Paravision 6.0.1. Mice were placed supine over a 2×2 surface receive array inside a volume coil. Respiration and body temperature were monitored and maintained at 30–40 bpm and 34–35°C, respectively. T₁-weighted RAREst dynamic contrast enhanced MRI of these mice was performed, with TR=200ms, TE=4.66ms, FOV=38.4 × 38.4 mm, 8 slices, 1mm slice thickness, and 192 × 192 matrix size. A baseline was established over three acquisitions, and mice were injected via tail vein catheter with a 0.050 mmol/kg solution of contrast agent, followed by a 100µl saline flush. For a 30g mouse, 60µl of contrast agent was injected manually via syringe over 3 seconds, and the 100µl saline flush was performed manually over 5 seconds. Injection success was determined in real-time via the absence of resistance to manual pressure on the plunger, and then confirmed retrospectively by enhancement of the renal artery at minutes 4 and 5 of the scan (Supporting Information Figure S1). Images were obtained at 1-minute temporal resolution for the next 57 minutes (Figure 1). Percent hepatic MRI signal enhancement was calculated for each time point based on a region of interest and normalized to baseline. Data in Table 1 is reported as both area-under-the-curve from baseline (AUC) and peak percent hepatic MRI signal enhancement. To confirm inhibition of human OATP1B1 and OATP1B3, mice also underwent DCE-MRI with Gd-EOB-DTPA an hour after injection of rifampicin in 5% DMSO at 5 mg/kg. To adjust for hepatic MRI signal enhancement resultant from blood pool, the hepatic MRI signal enhancement of wild-type FVB mice and chimeric mice was normalized using Oatp1A/1B knock-out mice as the baseline. Statistical analysis was done with a two-way ANOVA in Microsoft Excel.

Measurement of Contrast Agent Elimination

Single mouse metabolic cages from Techniplast were obtained through VWR. The mice were acclimated to cages for 24 hours before intravenous injection of contrast agents. Feces and urine samples were collected at 2, 4, 6, 12, 18, and 24 hours. Samples were dried, digested with 69% nitric acid from Sigma, and diluted further to 2% nitric acid. Gadolinium concentration was determined for each sample by inductively coupled plasma optical emission spectrometry (ICP-OES). Mass of gadolinium cleared per time point and percentage elimination via feces vs kidney urine were calculated. Enterohepatic circulation

for these contrast agents is negligible for Gd-EOB-DTPA (2.1%) and absent for Gd-BOPTA and Gd-DTPA⁹, and all three contrast agents have a half-life of 2 hours or less¹⁰. Thus, the proportion of contrast agent eliminated in feces vs urine over 24 hours can be used as an estimate for hepatic vs renal clearance. Statistical analysis was performed with a two-way ANOVA in Microsoft Excel.

Results

Imaging Studies

Figures 2a-c show plots of percent hepatic MRI signal enhancement for mice injected with one of either Gd-EOB-DTPA, Gd-BOPTA or Gd-DTPA, with the summarized and individual measurements shown in Table 1. Wild-type FVB mice exhibit rapid and sustained hepatic MRI signal enhancement following injection of hepatospecific agents Gd-EOB-DTPA (Figure 2a) and Gd-BOPTA (Figure 2b), with low hepatic MRI signal enhancement following the injection of non-specific Gd-DTPA (Figure 2c). In OATP1A/1b cluster knockout mice, hepatic MRI signal enhancement following injection of Gd-EOB-DTPA and Gd-BOPTA was diminished to 3.5% and 3.9% respectively relative to wild-type FVB mice, with this low hepatic signal enhancement likely due to blood pool enhancement, confirming the importance of hepatic OATPs for hepatospecific uptake. In chimeric mice, hepatic MRI signal enhancement following injection of Gd-EOB-DTPA was high and similar to that of the wild-type FVB mouse. In contrast, hepatic MRI signal enhancement following injection of Gd-BOPTA was low, reduced to 18% of the enhancement in wild-type mice, with a 'huge' effect size (Cohen's d) of ~ 4.5, demonstrating the robustness of these measurements even with a cohort of only 3 animals¹¹. Hepatic MRI signal enhancement following injection of Gd-DTPA was low in both wild-type FVB mice and chimeric mice, but higher than knockout mice, yet not in a statistically significant manner. AUC analysis in Figure 3a shows a non-significant difference between enhancement of the liver by Gd-EOB-DTPA in FVB mice vs chimeric mice, and Figure 3b shows a significant difference in hepatic MRI signal enhancement by Gd-BOPTA in wild-type FVB vs chimeric mice, with a 'huge' effect size (Cohen's d) of ~ 6, again, demonstrating the robustness of these measurements even with a cohort of only 3 animals. These summarized and individual measurements are shown in Table 2. Injection of rifampicin at 5 mg/kg to inhibit hepatic OATPs reduced AUC of hepatic signal enhancement by Gd-EOB-DTPA in chimeric mice to 5.2% relative to FVB mice (Figure 2d), confirming pharmacological responsiveness of transgenically expressed OATPs to known inhibitors.

Metabolic cages were used to perform elimination studies to compare physiological clearance of contrast agents with MRI studies, with results shown in Figure 4a-d. At 24 hours, in wild-type FVB mice, 79% of Gd-EOB-DTPA was recovered in fecal samples and 20% in urine (Table 2). In chimeric mice, this elimination profile was nearly identical to that measured in wild-type mice, with 81% elimination via feces and 19% via urine. Gd-BOPTA elimination in wild-type FVB mice was 83% via feces vs 16% in urine. This elimination profile was significantly altered in chimeric mice, with 32% elimination via feces and 67% via urine, with a 'huge' measured effect size (Cohen's d) of ~ 3. Gd-DTPA elimination in wild-type mice was 10% via feces and 89% via urine, with a similar profile in chimeric mice

(8.6% liver and 91% kidney). The detection of a small amount of gadolinium in feces of mice injected with Gd-DTPA was likely due to contact between urine and fecal samples prior to collection.

Discussion

Research and development of new contrast agents remains a very active area of research, and animal models that more closely mimic human systems are important for translation of findings. In this study, most significantly, we show that the organ elimination of Gd-EOB-DTPA, Gd-BOPTA, and Gd-DTPA in chimeric OATP1B1/1B3 knock in mice, in contrast to wild-type mice, closely recapitulates the organ clearance observed in humans¹, which in turn determines the measured hepatic MRI signal enhancement characteristics. Indeed, in chimeric OATP1B1/1B3 knock in mice, we measured high hepatic MRI signal enhancement and elimination of Gd-EOB-DTPA and low hepatic MRI signal enhancement and elimination of Gd-BOPTA. We previously verified that the hepatic MRI signal enhancement by Gd-EOB-DTPA and Gd-BOPTA in Oatp1A/1B cluster knock-out mice was abolished relative to wild-type FVB mice, showing the dependence of Gd-EOB-DTPA uptake on the Oatp1A/1B transporter family⁸. Since these mice were used to further engineer chimeric OATP1B1/1B3 knock-in mice, the human-mimicking hepatic MRI signal enhancement and organ clearance in the knock-in mice is due to the presence of human OATP1B1/1B3 proteins. Furthermore, pharmacological inhibition of OATP1B1/1B3 in chimeric mice by rifampicin is similar to humans, with rifampicin diminishing hepatic MRI signal enhancement by Gd-EOB-DTPA by 94.8%¹². Low P-values (Table 1) and ‘huge’ effect sizes (Cohen’s d) demonstrate the robustness of the study even with cohorts of 3 mice per group.

The elimination of contrast agents in wild-type FVB and chimeric OATP1B1/1B3 mice is in agreement with the enhancement profiles seen in MRI studies. In these studies, fecal recovery was used to approximate hepatic clearance and urine recovery to approximate renal clearance. Elimination profiles of Gd-EOB-DTPA or Gd-DTPA in wild-type FVB mice and chimeric OATP1B1/1B3 knock-in mice were statistically identical, supporting our imaging findings as well as previous literature that shows that Gd-EOB-DTPA is avidly taken up by both human and rodent OATPs, while Gd-DTPA has no affinity for these transporters¹³. There was a significant difference in elimination via feces vs urine between wild-type FVB mice and chimeric OATP1B1/1B3 knock-in mice for Gd-BOPTA. In the wild-type mice, the gadolinium was primarily recovered in the feces, with little recovery in the urine, indicating clearance mostly by the liver. In chimeric OATP1B1/1B3 knock-in mice injected with Gd-BOPTA, fecal recovery was diminished by a factor of 2.5, which reflects the three-fold reduction in AUC for enhancement seen between the two mouse models in imaging.

This animal model shows potential to not only expedite the bench-to-bedside process of contrast agent development, but also to decrease negative patient events in clinical trials for all pharmaceuticals. However, there are some caveats and opportunities for refinement. The use of the Cre and Lox mediated random transgenic insertion can lead to variable levels of protein expression. This could lead to some discrepancies between the ratio and absolute values of protein expression in mice relative to human systems. It may be possible to improve on this model using CRISPR¹⁴ mediated modification. Additionally, the ApoE

promoter was used to induce liver-restricted expression of human OATPS, while the native promoter is HNF1 α promoter. Thus, expression of the protein in transgenic mice could be moderated differently than in native systems. For example, it is suspected that HNF1 α expression and polymorphisms are involved in diabetes¹⁵, while ApoE is involved in Alzheimers¹⁶. Furthermore, there are some additional proteins that contribute to MRI contrast agent uptake in the liver, such as NTCP³. Investigation of mice with human NTCP instead of rodent NTCP may be necessary to further humanize this model. MRPs could also be humanized to mimic the efflux kinetics of human hepatocytes. Furthermore, the knock-out model leaves the mouse kidney bereft of OATP1A1, likely altering kidney clearance, so an improved model would also restore OATP function in the kidney. A final point to note is that only male mice were used in this study. While it is known that OATPs, and OATP1A1 in particular, have sex-based differences in expression, they would not emerge in this study¹⁷. However, it will be necessary to validate the sex-based expression of chimeric OATPs in this model, and plan experiments accordingly. Regardless of these caveats and problems, this current commercially available model is a distinct improvement on traditionally used wild-type models for development of MRI contrast agents for clinical use.

Conclusion

The OATP1B1/1B3 knock-in mouse accurately recapitulates organ clearance of FDA-approved hepatospecific MRI contrast agents to mimic that seen in human systems, making it a more appropriate model for the evaluation of contrast agents designed for medical imaging than wild-type mice. This mouse model could also be used to study potential imaging markers produced by OATP function during disease and after liver transplant. We suggest exploration and use of chimeric mouse models that humanize other systems to better screen contrast agents and small molecules for medical use.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ANOVA	Analysis of variance
ApoE	Apolipoprotein E
AUC	Area under the curve
DCE-MRI	Dynamic contrast-enhanced magnetic resonance imaging
ICP-OES	Inductively coupled plasma optical emission spectrometry
IV	Intravenous

NTCP	Na ⁺ -taurocholate cotransporting polypeptide
OATP	Organic anion-transporting polypeptide
RAREst	Rapid Acquisition with Refocused Echoes with short TE

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Figure 1:
DCE-MRI images of the mouse abdomen. (A) FVB animal before Gd-BOPTA injection, (B) FVB animal 27 minutes after Gd-BOPTA injection, (C) OATP1B1/1B3 knock-in animal before Gd-BOPTA injection, and (D) OATP1B1/1B3 knock-in animal 27 minutes after Gd-BOPTA injection.

Figure 2:

A) DCE-MRI curves for Gd-EOB-DTPA, B) Gd-BOPTA, C) Gd-DTPA, D) Gd-EOB-DTPA with and without rifampicin inhibition. Error bars are standard deviations from ROI analyses averaged within the entire group.

Figure 3:

AUC for hepatic MRI signal enhancement by A) Gd-EOB-DTPA, B) Gd-BOPTA, C) Gd-DTPA, D) Gd-EOB-DTPA with and without rifampicin inhibition. (*P<0.05) Error bars are standard deviations from AUC from baseline averaged within the entire group.

Figure 4:

Elimination of MRI contrast agent via urine and feces over 24 hours. Graphs (A) and (D) show elimination pattern of Gd-EOB-DTPA over time for both wild-type and chimeric mice. Graphs (B) and (E) showed elimination of Gd-BOPTA by liver in chimeric vs wild-type mice. Graphs (C) and (F) show elimination patterns of Gd-DTPA in wild-type and chimeric mice.

Table 1:

Peak hepatic MRI signal enhancement with Gd-EOB-DTPA and Gd-BOPTA in wild-type FVB mice, OATP1A/1B cluster knock-out mice, and OATP1B1/1B3 knock-in mice with and without rifampicin. Data are normalized as a percentage of hepatic MRI signal enhancement in wild-type FVB mice for each agent. N=3 animals for all measurements

		Mouse	FVB (stdev)	OATP1A/ 1B cluster knock-out (stdev)	OATP1B1/1B3 knock-in (stdev)	OATP1B1/1B3 knock-in with rifampicin (stdev)
Peak signal enhancement	Gd-EOB-DTPA	Mean	235%[#] (28.8%)	20.2% (10.8%)	280% (35.5%)	36.5%[#] (21.8%)
		1	220%	11.6%	367%	28.3%
		2	234%	29.6%	267%	19.3%
		3	251%	19.5%	207%	61.8%
	Gd-BOPTA	Mean	284%^{*†} (24.3%)	32.2%[*] (24.1%)	112%[†] (37.8%)	-
		1	256%	16.6%	118%	-
		2	318%	52.6%	162%	
		3	278%	27.2%	57%	

^{*,#}=P<0.01

[†]=P<0.02.

Table 2:

AUC of hepatic MRI signal enhancement with Gd-EOB-DTPA and Gd-BOPTA in wild-type FVB mice, OATP1A/1B cluster knock-out mice, and OATP1B1/1B3 knock-in mice with and without rifampicin. Data are normalized as a percentage of hepatic MRI signal enhancement in wild-type FVB mice for each agent. N=3 animals for all measurements

		FVB (stdev)	Mouse	OATP1A/1B cluster knock-out (stdev)	OATP1B1/1B3 knock-in (stdev)	OATP1B1/1B3 knock-in with rifampicin (stdev)
AUC relative to FVB	Gd-EOB-DTPA	100% [#] (19%)	Mean	3.5% (0.88%)	134% (17%)	5.2%[#] (4.1%)
			1	3.5%	124%	3.1%
			2	4.3%	151%	2.4%
			3	2.6%	131%	10%
	Gd-BOPTA	100% ^{*†} (13%)	Mean	3.9%[*] (1.6%)	22%[†] (4.7%)	-
			1	2.6%	23%	-
			2	5.7%	26%	
			3	3.2%	18%	

^{*,#}=P<0.01

[†]=P<0.02.

Table 3:

Mean percent elimination of contrast agents via feces and urine in wild-type FVB mice and OATP1B1/1B3 knock-in mice.

	FVB Mice		OATP1B1/1B3 Knock-In Mice	
	Feces (stdev)	Urine (stdev)	Feces (stdev)	Urine (stdev)
Gd-EOB-DTPA	80% (6%)	20% (6%)	81% (12%)	19% (12%)
Gd-BOPTA	* 83% (14%)	* 17% (14%)	* 32% (12%)	* 68% (12%)
Gd-DTPA	11% (8%)	90% (8%)	9% (4%)	91% (4%)

*
P<0.05