Scintica: INSTRUMENTATION

WEBINAR: Novel Nanoparticle-based Contrast Agents for Preclinical MRI Studies at 1T: Research Applications in Placental Disorders

Questions and answers from the September 24, 2019 webinar titled "Novel Nanoparticle-based Contrast Agents for Preclinical MRI Studies at 1T: Research Applications in Placental Disorders"

This document includes questions we received and answered during the webinar, as well as those that we did not have time to address.

1. **Most preclinical MRI is performed at higher field strengths due to the small size of the animals and the small field of views required for high resolution imaging. Could you talk about the benefits of using higher field system (e.g. 7T or even 9.4T) for these applications?**

   The primary difference between high field and low field strength systems is signal quality. Generally speaking, image resolution is lower with low field systems and higher with higher field strength systems. A big point when looking at the higher field strength systems is that it is possible to use more complicated imaging sequences, such as diffusion weighted imaging (DWI), diffusion tensor imaging (DTI) and other sequences that allow you to get additional information about the target system or the features of interest. With that said, when considering the performance of 1T scanners, the relaxivity profile for agents is optimized at those lower field strengths, so this is advantageous. Also, having the equipment in the room enables considerably high throughput for experiments because it so easy to access and does not require an engineer or physicist to operate.

2. **How do you apply the contrast agent?**

   A pre-scan is first taken, the contrast is administered primarily through tail vein catheterization, and then the image is taken. For dynamic contrast enhanced (DCE) imaging, Aspect's system has a port you can attach to the catheter and use it to administer other agents, particularly those that don't have as long of a half-life as the liposomal-Gadolinium agents I discussed in my presentation.

3. **Can you talk a bit more about throughput and how many animals you image per day?**

   We are able to analyze 8-10 mice per hour using the T2-weighted fast-spin echo protocol discussed in the presentation. 300um resolution in plane is relatively sufficient for our applications. If you go up to a 30-40-minute scan protocol you can get to about 100um resolution in plane. 200um isotropic is probably the limit at 1T, which is a difference between the higher strength systems versus lower strength systems.

4. **What is the survival rate of the animals that were injected with your agents?**

   I don't have the exact data on me right now for that, but I can say we have a very low fatality rate with injection for this agent. Most deaths are due to handling or health of the animals, particularly the cancer models. We have done toxicology studies ((Tanfium et al, "Determination of the fate of intravenously administered Gd(III)DOTA-DSPE-based liposomal contrast agent in a mouse model", ISMRM 2019)) and we've looked at the clearance mechanism. If you want to look at some papers from my group, particularly Eric Tanifum, they have a conference abstract recently out for the International Society of Magnetic Resonance in Medicine Annual Meeting where they look at the profile for this agent, where it accumulates, how long it takes to accumulate, and the methods for clearance. A publication on this data is in preparation. Briefly, however, it is spleen, liver, and biliary excretion

5. **Why is it so important for these blood-pool agents to have long circulation?**

   For conventional agents, you often have windows on the order of minutes (10 minutes or so) where you'll have the agent in the field of view, which is suitable for dynamic contrast imaging, where you're continuously injecting and taking real time images. However, when you look at imaging the way we're doing, which we think will be more valuable for clinical use as well (administration and then post-contrast imaging), then having long circulation is very critical. When looking at different vascular compartments, it being a true blood-pool agent is important as signal enhancement is either directly proportional (in the case of CT) or indirectly proportional (in the case of MRI) to the vascular perfusion.

6. **Approximately what size were the nanoparticles that were chelated in the contrast agent? If different sizes were used, were there discernable differences in subject viability when injecting the contrast agent?**

   I don't have that information directly on hand, but if you look at the publications for our placental imaging applications (Badachhape et al, "Pre-clinical magnetic resonance imaging of retroplacental clear space throughout gestation", Placenta 2019), that information will be in the methods and materials sections. I don't remember exactly the nanometer size off the top of my head. The size mostly has impacts on kinematics and clearance mechanisms for it, which is important for vascular imaging. Excerpt from my paper: "The mean liposome size in the final formulation, determined by dynamic light scattering (DLS), was 122.23±0.27 nm with a poly-dispersity index of less than 0.15."

7. **What is the optimal time point for imaging post injection of the liposomal-gadolinium?**

   We often try to image within an hour. I was just talking about throughput for non-contrast applications. For contrast applications, since we are doing tail vein injections, which do require a certain amount of expertise, we will typically image within the hour of injecting these agents. Another valuable method, given just how long-lasting and the circulatory properties of these, is a delayed post-contrast scan, maybe on the order of days after we've injected the contrast when we look at tumours. The thinking there is once it's cleared out of the circulation, any contrast that has accumulated in the periphery of the tumour is likely a result of a leak in tumour vasculature, which could be useful for better understanding angiogenesis and different leaky vessels.

8. **With the long T1 relaxation of the gadolinium contrast agent, how would you propose the scan protocol of an analogous human scan with a feasible scan duration? In addition, how would you propose dealing with respiratory motion in humans?**

   For relatively long scan protocols, you have gating techniques which are often used to deal with respiratory motion in humans. Especially if you're going to be visualizing in the upper abdomen area where you have to worry about lung or cardiac motion, gating is extremely important, particularly for looking at relatively small vessels as motion artifact can impact your resolution for that. Regarding a scan protocol for analogous human scan with feasible scan duration, I would probably consider still scanning within 1 to 2hours despite the relatively long half-life for this agent. We are still working on the translational impact for these agents, and while we do anticipate using these in the clinic, there is still a

long way to go, such as thinking about how they're going to be used and what scan protocols are going to be used. But I would consider about a 30-minute protocol for your average anatomical scans, your different contrast-enhanced subsets of scans, and potentially other types of scans like FLAIR (fluid-attenuated inversion recovery) imaging and DWI imaging which could be very useful in conjunction with contrast-enhanced imaging. As an aside, you can perform DWI and approximate inversion recovery sequences using the Aspect scanners.

9. **How do you create the accreta model (adherent placenta)? What is RPCS volume?**

   That is the logical next step for this placental accreta study. We currently have several models that we are working on that are also documented in the literature as demonstrating accreta models (Sliz et al, "Gab3 is required for IL-2- and IL-15-induced NK cell expansion and limits trophoblast invasion during pregnancy", Science Immunology 2019). I recommend looking in the literature to learn more about those. We also are looking at potential surgical models, wire-scratch models or potentially even limited C-section delivery models, to try and see if we can replicate adherent placenta through those models.

   The RPCS volume is relatively small, on the order of 8-20 mm^3 according to the segmentation we performed in ITK-SNAP and confirmed with histopathology. We would expect that RPCS volume to be at least lowered in an accreta model, but I think what's going to be more likely for diagnosing accreta is looking at infiltration across that junctional zone. So, looking for disruption of that space, because in those images we see a clear crescent pattern between the placenta and endometrial wall that we think is going to likely be disrupted in cases of accreta when we look at those models.

10. **What is the liposomal half-life in tissue? Can you use it for steady state venous imaging?**

    We can use it for venous imaging. I don't have the half-life in tissue off the top of my head, but I think I will depend on the fractional blood volume for the tissue at hand. For example, in the placenta or tumours it will probably have a half-life closer to the 18-hour mark. I'll refer again to the Eric Tanifum conference paper that looks at the liposomal-gadolinium half-life in various tissue response.

11. **Since the agent extravasates into tumor tissue periphery, can the gadolinium agent potentially load drugs for therapeutics?**

    What we always have to be worried about with coupling additional molecules or chelating additional therapies is clearance mechanisms, the size, and where it is going to actually be able to get into. One thing we do often do in applications with agent that our lab has published (Tanifum et al., "Intravenous Delivery of Targeted Liposomes to Amyloid-β Pathology in APP/PSEN1 Transgenic Mice", PLOS One 2012), is we've added targeting ligands to these liposomal agents for looking at biomarkers or other features of interest. For example, regarding Alzheimer's Tau imaging, we have worked on Tau ligands that we can use for looking at those agents. As for treatments, I'd need to talk to some of the chemically inclined members of our group for that. We would have to be worried about the overall stability of the agent for adding therapeutics, but I think theoretically it's probably possible for some of these agents.

12. **Outside of placental and tumor imaging, what are other applications for liposomal-gadolinium?**

    Cardiac imaging, looking at leak patterns or disruption of larger vessels (i.e. descending aorta) are examples. We are also looking at Alzheimer's disease and targeting other pathology or biomarkers in the brain. The best part about this platform is its stability, which allows us to tinker and think about lots of different applications for these molecules, and I think that will lead to a large number of preclinical applications and hopefully eventual clinical applications. I know that a lot of other research groups have looked at liposomal coupled agents to use for both imaging and therapeutic needs. I think there are a large number of applications that can be considered.

13. **Do the liposomes enter cells and release the gadolinium inside cells, or does it stay within the tissue microenvironment? For instance, in tumors.**

    This is mostly extracellular deposition, extracellular binding and accumulation. However, there are some intracellular applications.

14. **Given the safety concerns regarding gadolinium use in the clinic, what is the translational potential for the liposomal-gadolinium agents shown in this talk?**

    Gadolinium has come under fire and increased scrutiny based on new studies about inflammation and other problems that have occurred as a result of gadolinium exposure. These issues have motivated us to ensure it does not cross

the placental barrier and risk fetal exposure to gadolinium. However, even with those guarantees, we still always have to be mindful of gadolinium use and of these issues. We use gadolinium for these agents simply because it has such high signal enhancement. But there's no reason we can't think about other contrast mechanisms, such as iron oxide particles or other types of molecules that have started to emerge like ferumoxytol and its off-label use for vascular imaging. I think that these liposomal agents still have strong clinical translation potential, however, because of the lack of exposure to the fetus and because with any sort of contrast enhanced imaging mechanism, you always have to weigh the trade-off between cost and benefit. In other words, what is the benefit to the patient and what is the potential for being able to diagnose and understand what could be happening versus the cost of inflammation or other side effects from gadolinium. So, when thinking about the translational impact for contrast agents, we have to be careful of that trade-off. I believe that for the agents we talked about today, the benefits still outweigh the cost for potential issues with gadolinium, as long as the side effects and precautions are laid out at the beginning.

15. Why do you designate day 10 as 10.5 and day 12 as 12.5 etc. when referring to time points where you collect data?

In a preclinical model of gestation, things happen very quickly – we're talking about a total time period of 21 days. If I image in the morning and then image in the afternoon, there will be appreciable difference in gestational development that you can see in these models. So, we'll often do a 0.5 to denote the flexibility and ambiguity in the actual time point we're looking at. We've been talking about the timescale of these experiments because of the gadolinium agent we're using, but you also have to be really careful about your imaging and be relatively efficient because when scanning all day, there's going to be actual changes. So, I try to have a relatively quick scan protocol in the order of about 30 or so minutes, even for these contrast enhanced scan protocols, which is really important for doing preclinical imaging of pregnancy.

16. What analysis software are you using?

OsiriX (MAC OS) for image presentation. ITK-SNAP for image segmentation (any OS)

17. How do the agents exit the body after localizing in the spleen and liver?

Biliary excretion.

18. How is the stability of the particle?

For shelf life, our lab has seen these agents remain stable for a long period of time (order of years). This is because the formulation uses saturated lipids which keeps the nanoparticle intact in solution. In vivo, the MRI contrast agent has a half life of ~18 hours and our lab has not seen any component disruption in our studies. We have also confirmed these findings with ICP of experimental tissue. See Badachhape et al, Placenta 2019 for additional information about the composition of these agents.

19. Is gadolinium chelated or not inside the liposomes?

Gadolinium is chelated to the lipid bilayer of the nanoparticle (see slide 3 of my presentation for a diagram).