Live Animal Imaging: Challenges & Longitudinal Analysis
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Introduction

This presentation will focus on imaging in translational animal models and longitudinal data analysis.   One of the great advantages of non-invasive imaging techniques is the ability to repeat measurements thus reducing the number of animals required for longitudinal studies and decreasing inter-animal variability by using the same animal as its own control.   In this sense, the noninvasive property of MRI is enormously valuable for serial interrogation of disease status, allowing development of clinically relevant, statistically robust tools for disease profiles without the use of expanded cohorts of animals or resources. Moreover, as a general platform tool, methods developed in the preclinical stages can be directly adapted to the clinic. This ability to connect preclinical and clinical applications can facilitate the practice of translational medicine, wherein the tools used to gauge the preclinical efficacy of a therapy may also be used in clinical development and treatment plans.

For drug development, MRI is particularly well suited to study in vivo physiology: MRI is noninvasive, is inherently 3D, and has high spatial resolution. Additionally, there are a variety of physiologically relevant contrasts such that numerous indices can be developed that correlate with therapeutic modulations of a test drug with high spatial accuracy and with novel information content.

Some of the challenges inherent in translational animal models are to mimic human physiology, pathophysiology, and response to treatment, and to have imaging methods that can be applied across species in an efficient manner.   In addition, there are numerous challenges in the analysis of longitudinal imaging data to determine the best markers of disease progression and treatment responses. Excellent recent reviews of translational animal models in drug discovery can be found in the references (1,2).

Animal Models

Translation animal models can range from genetically altered mouse models to non-human primates (NHP's).   Other commonly used animal models in translational imaging studies include rat ,guinea pig, rabbit and dog.   Each animal model can have characteristics that are best suited to human translation.

Transgenic mouse models: Transgenic animals are widely used as experimental models to perform phenotyping and for testing in biomedical research. Transgenic manipulation of mice has created phenotypes with the potential of linking specific genes to molecular, cellular, and organ functions. Application of the transgenic or gene ''knock-out'' mouse mutant technology to studies of the biochemistry and physiology of the brain, heart, musculosketetal, and metabolic systems has historically been through necropsy studies, which require the sacrificing of many animals and the performance of tedious assays which can be avoided with longitudinal imaging methods.

Rodent models: Other rodent models such as rat and guinea pig are often preferred for imaging studies relative to mice due to their larger size (hence less stringent image resolution constraints and easier surgical manipulation).

Large animal models: Non-human primate (NHP) models for both normative and perturbed biology are uniquely suitable for translational imaging research. Genetically, NHPs share up to 99% of the human genome and thus are more likely to have similar pathology and treatment responses to humans. Primates typically studied with imaging can vary greatly in size ranging from small marmosets (<500g) to cynomolgus (3-6kg) to rhesus (5-10kg) which present a wide range of imaging challenges.

Physiology

Anesthesia: To perform in vivo imaging requires maintaining and monitoring the physiology of the animal during imaging sessions. Anesthesia affords more control over the animal and a recent review discusses usage of different anesthetics for MRI applications (3). In general, inhalation anesthetics, such as isoflurane and halothane, are employed because they are easy to control and are fairly gentle to the animal. Induction in rodents is typically done at 3 to 4%, whereas maintenance is at 1-2% isoflurane in 100% oxygen (4). For longer imaging studies, animals can be injected intraperitoneally with saline after induction to prevent dehydration.

Monitoring: The monitoring of the physiological state can involve any or all of the following: ECG, temperature, ventilation, exhaled CO_2, and blood pressure depending on the animal model and study type. Generally speaking large animal models such as dog and primate require more accurate monitoring than rodents. Because the monitoring of the animal will take place inside a magnetic field, all connections need to be nonmagnetic. Furthermore, to prevent interference from the gradients, either fiberoptic connections or analog filters are necessary.

fMRI: Functional imaging often requires more stringent physiological control compared to anatomic and compositional imaging methods in order to establish a stable and reproducible physiologic state and to preserve accurate neurovascular responses. In many species, this requires different injectible anesthetics such as; alpha-chloralose, propofol, and dormitor or significantly lower doses of inhalational anesthesia. More recently, it has been shown that fMRI can be performed in conscious animals with appropriate training to tolerate the MR environment (5, 6).

Imaging methods

Small animal: To scale MRI down to small animals such as mice, ranging in size from 25 to 40g, requires a number of changes. Because of decreased voxel size, higher magnetic field strengths are better suited as useful signal scales with field strength; 7, 9.4, and 11.7 T are usual field strengths, as compared with 1.5 or 3 T for clinical MRIs. The net result is the ability to image less than a hundred micrometers, instead of the usual millimeter resolution on clinical scanners. Nevertheless, mouse imaging can be performed in a limited manner on clinical scanners when high field options are not available.

Multiple mouse imaging: When performing biological research, the ability to find conclusive results requires studying multiple subjects—on the order of dozens—to improve the statistics of the experiment. From another point of view, random mutagenesis programs can create upwards of thousands of mice per year. With such numbers, using MRI as a phenotyping tool can be problematic because of imaging times (typically 1min-30min). In pharmacologic applications, large animal numbers are often

needed to account for intrinsic biological variability to a drug response. For higher throughput it is possible to image multiple mice, thereby increasing throughput. This can be accomplished to varying degree by developing multi animal holders with conventional image acquisition (7) or by using multi receiver MR coils to acquire images from multiple animals in the same acquisition time as for a single animal (4,7,8). Prototypical four-mouse imaging systems are now commercially available.

Large animal: Typically large animal imaging needs to be performed on a clinical scanner which has sufficient bore size to accommodate larger animals and associated physiological monitoring equipment.   This presents the challenge in modifying human imaging protocols to accommodate smaller organ sizes.   For example, brain imaging in NHP models must be adjusted for the comparatively small brain size relative to humans (2- to 200-fold smaller volume, depending on the species). (9)

Structural imaging research offers excellent translational benefits when non-human primate (NHP) models are employed. Clinical protocols such as MPRAGE and TrueFisp can be optimized for structural imaging. In addition, computational tools that have been developed for the analysis of human structural images can be applied to the NHP studies. These included removal of non-brain tissues, correction for RF inhomogeneity, spatial normalization, and the building of an optimized target anatomic atlas.

Reverse translation: In many cases human imaging protocols can be more sophisticated than the small animal analogs since they have been developed for patients over many years and corresponding animal models have only been developed more recently. Examples of this would include cardiovascular imaging of atherosclerotic plaque (10) or cartilage imaging in osteoarthritis(11).   In these cases the challenge is to develop small animal analogs that can probe similar pathophysiology in reasonable imaging times.

Identification of Imaging Biomarker

Given the large number of possible imaging biomarkers available by MR there is the additional challenge to choose the most appropriate maker. Once the marker is identified and shown to be robust in a small batch of animals, the issue of quantitation needs to be seriously reviewed (1). It is important to recognize that several indices can be devised to capture different features of a disease marker. For example, the complexity of the marker may range from lesion volume to modeling the temporal–spatial progression of ischemia in specific cerebral regions. In the development of imaging markers it is important to account for sources of variability, sensitivity of the index to modulation by drug candidates; and feasibility and resources needed to calculate the index in a high-throughput mode.   It is also important to consider that the outcome measure should be acceptable not only to physicists but also to collaborating chemists and biologists.

Longitudinal analysis:

The longitudinal analysis of imaging data can be done on both a regional and voxelwise manner.   Typical regional analysis of longitudinal biomarker data is performed using statistical models such as Repeated Measures Anova for within group comparisons and Mixed Effect Models that incorporate both within group and between group comparisons (12,13).   Figure 1 shows an example of longitudinal monitoring of

brain volume in a transgenic mouse model of Alzheimer's disease compared to wildtype mice with and without treatment with doxycycline (14). A mixed effects model can be used to show differences in atrophy rates both within and between study groups indicating both a difference between wildtype (WW) and induced transgenic mice (IND TG) as well as positive treatment effect (prevention of brain atrophy) in the transgenic mice at 24 weeks. Additional examples of longitudinal image analysis to be discussed will include, aging, inflammatory disease, tumor growth, and drug responses.
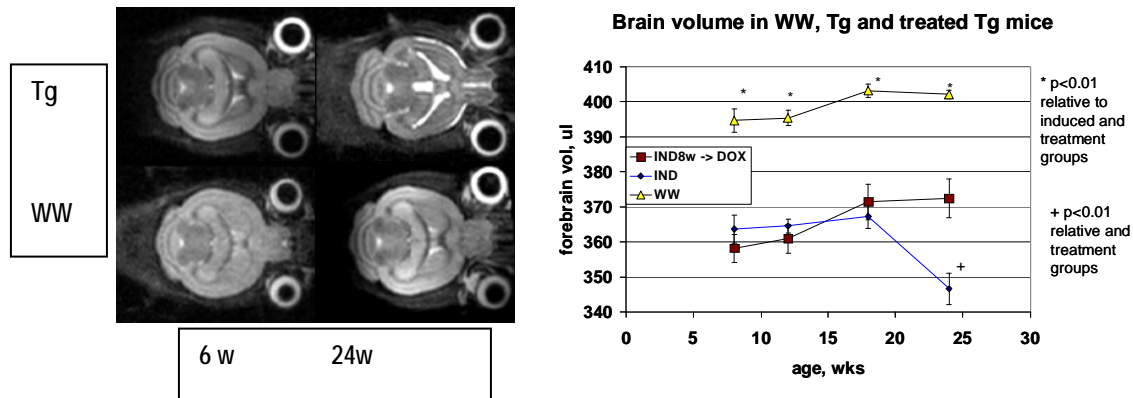

Brain volume in WW, Tg and treated Tg mice

Figure 1: Longitudinal progression of brain loss in Tg mouse model of Alzheimer's disease

Similar analyses can be performed on imaging data spatially in a voxelwise manner. These voxel morphometry techniques (15) are often applied to determine regional atrophy rates in brain for the longitudinal study of neurodegenerative disorders such as Alzheimer's and Huntington's (16) disease.

Summary

Animal imaging by MRI has led to a rapid progress in the variety of ways disease biology can be monitored non-invasively in living animals. These advances have increased our knowledge of disease dynamics and refined the design of effective therapeutic interventions in a true translational manner. A constant challenge in translational imaging will be the refinement of animals models to best represent human pathophysiology. There is a continued need for improved animal models in oncology (tumor models), cardiovascular disease (atherosclerotic plaque), neurodegenerative disease (Alzheimer's) , and inflammatory disease (arthrtis) to name a few. In addition there is the challenge to translate animal imaging protocols into the clinical setting and to reverse translate many existing clinical imaging into small animal applications as new models develop. These new protocols need to be standardized when possible in order to compare experimental results between research groups. Ultimately, it is expected that the continued application of non-invasive imaging in animal models will result in improved patient care and lead to patient-specific therapies with greater efficacies and fewer side effects.

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