Magnetic Resonance Imaging of Denervation-Induced Muscle Atrophy and the Effects of Clenbuterol in the Rat

M. D. Cockman, M. B. Jones, M. C. Prenger and R. J. Sheldon, Procter & Gamble Pharmaceuticals, Mason, Ohio, USA

INTRODUCTION
Pharmaceutical studies directed toward discovering therapies for muscle atrophy depend on testing in animal models. Currently, pharmaceutical efficacy is assessed by dissection of muscles of interest and determination of their wet weight. This approach is labor intensive and requires large numbers of animals for multi-day time course studies. Clearly, a rapid and non-terminal means of assessing muscle loss or gain is highly desirable.

MRI is ideal for visualizing muscle. Multislice or 3D acquisition allows quantification of muscle cross-sectional areas and volumes. The non-destructive nature of MRI allows multiple evaluations of the same animal so changes over time can be followed. Hence, MRI allows the observation of the atrophy process and pharmaceutical intervention over time, information which can supplement pharmaceutical mechanism-of-action studies.

EXPERIMENTAL
Surgical model. We used adult, female Sprague-Dawley rats (230-270 g, Charles River Laboratories) to create a rat sciatic-denervation model of muscle atrophy (1). On Day 0, each rat was anesthetized and prepped for surgery. A small incision was made ~2 cm proximal to the knee on the lateral side of the leg. The muscles were separated, the sciatic nerve was lifted out with a surgical hook, and a 3-5 mm piece of the nerve was removed. The incision was closed and the rats remained in normal housing conditions for the duration of the study.

MR Imaging Protocol. MR imaging was performed on a Biospec MSLXI 1.47 Tesla Imaging Spectrometer (Bruker Medical, Inc.) using steady-state gradient-echo imaging protocols. Each rat was anesthetized with isoflurane vapor and positioned prone in a cradle with the leg or legs dangling through a 35 mm hole surrounded by a transceive coil (Morris Instruments). The orientation of the leg was not critical since the imaging protocol incorporated the acquisition of multiple localizer images. We used these to identify bony landmarks, and so acquisition of the slices to be used for analysis could be done reproducibly.

MR Image Analysis. Analysis was done using the ParaVision Xtip package (Bruker Medical, Inc.) Each cross-sectional area (CSA) measurement was made using manual segmentation to outline the region-of-interest with a mouse-driven cursor. The software automatically reported the CSA. Volumes were derived by summing the CSAs obtained from different slices and multiplying by the slice separation.

RESULTS and DISCUSSION
We first conducted a study to verify that MRI could qualitatively track atrophy in the rat sciatic-denervation model using a 3D volume imaging approach. The study was successful; however, the 3D protocol was too time-consuming (~1 animal / hour) to allow a reasonable animal throughput of 3-4 animals / hour. Hence we next conducted a Method Optimization study in an effort to speed up the acquisition and analysis. As shown by the graph, a single cross-sectional area (2D CSA) obtained near the muscle belly did a good job of estimating muscle mass.

Using MRI-Derived Cross-Sectional Areas to Quantify Pharmaceutical Effects in Muscle. Based on the results of the Method Optimization study, we next used the 2D CSA approach to the quantify the effects of a β2-adrenergic agonist, clenbuterol, in the rat denervation model. Clenbuterol is a benchmark compound which is known to produce hypertrophy in normal muscle and an anti-atrophy effect in denervated muscle. We measured 2D CSA over 28 days at 9 timepoints. There were 3 rat populations: sham-operated control (n=6), denervated (n=8), and denervated + clenbuterol (1 mg/kg/day, sc, n=8).

Typical images are shown below. This study showed that MRI can identify the onset and progression of clenbuterol therapy in the rat denervation model. However, when we compared these results with those from previous studies in which ex-vivo mass was the endpoint, we found that CSA from MRI overpredicted the efficacy of clenbuterol.

Using MRI-derived Volumes to Estimate Pharmaceutical Efficacy. We observed that single slice CSA at a fixed landmark biases the results in a group-specific manner, probably because muscle atrophy and the effects of clenbuterol are not uniform throughout the lower leg (1, 2). We hypothesized that volume measurements would capture the non-uniformity and provide more accurate estimates of efficacy. We conducted two additional studies and found that muscle volumes produced efficacy estimates more similar to those obtained from mass. For example, at Day 14, we found 37 ± 7%, 67 ± 11%, and 46 ± 10% efficacy (mean ± sem) from mass, CSA, and volume, respectively. With the mass results as the standard, note that CSA again overpredicted efficacy.

SUMMARY
MRI allows one to follow the time course of pharmaceutical therapy without requiring a large number of animals and avoids the need for dissection. Animal throughput is comparable to dissection without the need for sacrifice. Muscle volume estimates are needed to accurately assess pharmaceutical efficacy.

References