

A Non -invasive Assay of Matrix Metalloproteinase Induced Musculoskeletal Syndrome in Rats using High Resolution Magnetic Resonance Imaging.

R. P. Kennan¹, C. Keohane², J. Jackson², R. Hajdu², D. S. Williams³, S. Miller Mandala², H. Liu¹

¹Imaging, Merck & Co. Inc, Rahway, NJ, United States, ²Immunology, Merck & Co. Inc, Rahway, NJ, United States, ³Imaging, Merck & Co. Inc, West Point, Pa, United States

Introduction: Matrix metalloproteinases (MMPs) are considered excellent targets for drug development for arthritis and cancer, however the clinical utility of broad-spectrum MMP inhibitors has been restricted by a musculoskeletal side effect observed in humans and animal models (1). Currently, these MMP induced side effects can be characterized by clinical pain evaluations in patients (2) and post mortem histologic analysis in animal models (1). The objective of this study was to develop a non-invasive assay based on magnetic resonance imaging to characterize pathologic changes in a rat model of broad-spectrum MMP-induced musculoskeletal syndrome (MSS). Image based MSS biomarkers included mean growth plate thickness, synovial fluid volume and fat pad composition.

Animal Model: Experiments were performed on 18 male Lewis rats at an age of 10 weeks. Subcutaneous osmotic pumps were implanted to deliver the broad band MMP inhibitor marimastat at a constant rate over a 2 week period. In the first experiment vehicle infusion (N=6) was compared to the MMP inhibitor marimastat (N=6) at day 14 post infusion at doses of 30mg (N=4) and 10mg (N=2). In a second group (N=6), a 30mg marimastat infusion was evaluated serially over 2 weeks to determine the temporal progression of MSS. For comparison with the MRI results, behavioral evaluations were performed and clinical scores pain scores were assigned on a daily basis over the 2 week infusion period.

MRI: Imaging was performed on a 9.4 Tesla horizontal bore MR imaging systems (Bruker, Karlsruhe Germany). Rats were anesthetized through a breathing mask with 1.5% isoflurane mixed with oxygen. Rodent knees were imaged using a 2.5cm diameter single turn solenoid coil in which the knee was stretched perpendicularly to the body of the rat. The imaging protocol included high resolution 3D T1-w gradient echo imaging to visualize growth plate expansion and articular cartilage (res = 256x192x32, FOV= 15x15x8mm, TR=100msec, TE=3msec, flip angle ~30 degrees), and multi slice 2D fast spin echo imaging (res = 256x192, FOV = 25x25mm, slice thickness =0.6mm, 11 slices, TR=4sec, TE_{eff}=25msec, RARE factor = 4) with and without fat suppression in order to visualize growth plate thickness as well as MMP induced fluid and fat changes. Images were segmented to determine synovial fluid volume, growth plate thickness, and fat pad composition.

Results: MRI showed growth plate and synovial fluid increases as early as day 2 post infusion, well before clinical signs were present. This is illustrated in figures 1 and 2 which growth plate enlargement and significant synovitis which also leads to fat reduction in the fat pad. Over the 2 week period, the growth plate increased approximately linearly with time showing a 5-fold increase from 0.4±0.05mm to 2.2±0.2mm which was in qualitative agreement with previous histologic studies in the same model (1). Synovial fluid buildup in the knee accumulated supra-linearly from 1±0.4µl to 12±2µl at day 13 and the timecourse appeared to be more closely correlated with the onset of behavioral symptoms. In summary, MRI provides an important means for monitoring dose and time dependence of MSS that can be directly translated to future human studies.

References:

1. Renkiewicz R., Luping L., Lesch C., Sun X, Devalaraja R, Cody T, Kaldjian E., Welgus H, Barag V, ARTHRITIS & RHEUMATISM 48(6), 2003, pp 1742–1749
2. Hutchinson JW, Tierney GM, Parsons SL, Davis TR. J Bone Joint Surg Br 1998;80:907–8.

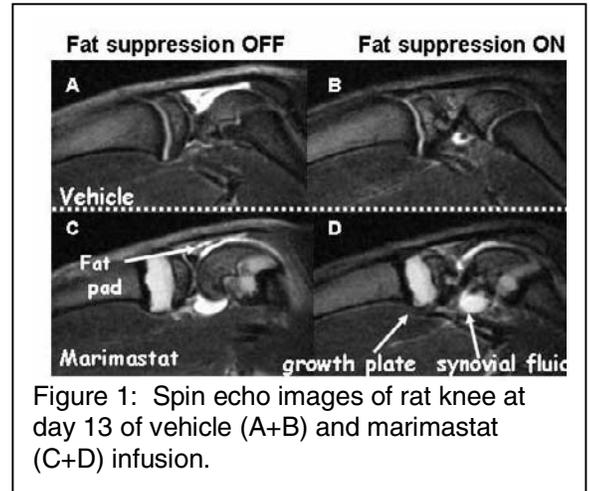


Figure 1: Spin echo images of rat knee at day 13 of vehicle (A+B) and marimastat (C+D) infusion.

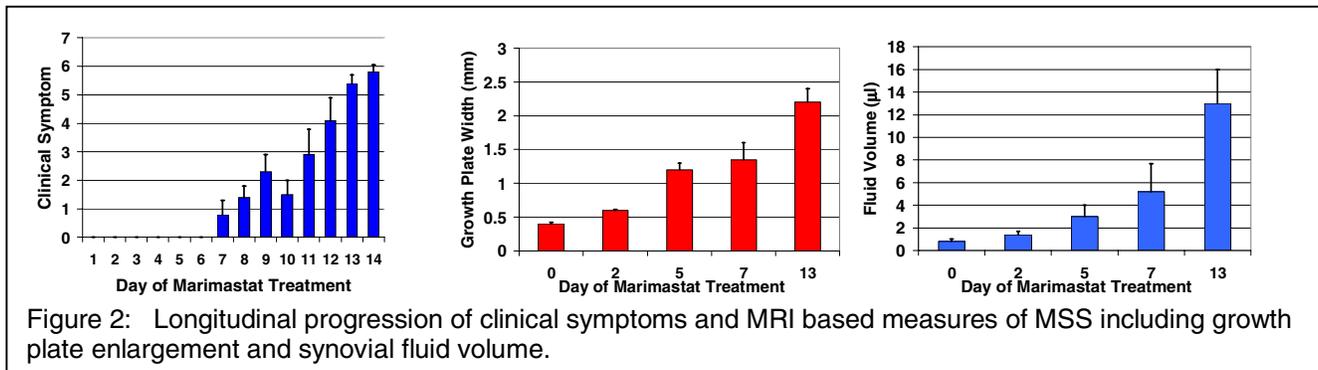


Figure 2: Longitudinal progression of clinical symptoms and MRI based measures of MSS including growth plate enlargement and synovial fluid volume.