Osteoarthritis and Cartilage xxx (2016) 1–9

Osteoarthritis and Cartilage

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Quantitative pre-clinical screening of therapeutics for joint diseases using contrast enhanced micro-computed tomography

N.J. Willett † [a], T. Thote ‡ [a], M. Hart §, S. Moran ‡, R.E. Guldberg †, R. Kamath § *

† George W. Woodruff School of Mechanical Engineering, Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, USA
‡ Wallace H. Coulter Department of Biomedical Engineering, Parker H. Petit Institute for Bioengineering and Bioscience, Georgia Institute of Technology, USA
§ AbbVie Bioresearch Center, Worcester, MA, USA

ARTICLE INFO

Article history:
Received 20 July 2015
Accepted 27 April 2016

Keywords:
MicroCT
Therapeutics
Osteoarthritis
Pre-clinical
Histopathology

SUMMARY

Objective: The development of effective therapies for cartilage protection has been limited by a lack of efficient quantitative cartilage imaging modalities in pre-clinical *in vivo* models. Our objectives were two-fold: first, to validate a new contrast-enhanced 3D imaging analysis technique, equilibrium partitioning of an ionic contrast agent-micro computed tomography (EPIC-μCT), in a rat medial meniscal transection (MMT) osteoarthritis (OA) model; and second, to quantitatively assess the sensitivity of EPIC-μCT to detect the effects of matrix metalloproteinase inhibitor (MMPi) therapy on cartilage degeneration.
Methods: Rats underwent MMT surgery and tissues were harvested at 1, 2, and 3 weeks post-surgery or rats received an MMPi or vehicle treatment and tissues harvested 3 weeks post-surgery. Parameters of disease progression were evaluated using histopathology and EPIC-μCT. Correlations and power analyses were performed to compare the techniques.
Results: EPIC-μCT was shown to provide simultaneous 3D quantification of multiple parameters, including cartilage degeneration and osteophyte formation. In MMT animals treated with MMPi, OA progression was attenuated, as measured by 3D parameters such as lesion volume and osteophyte size. A *post-hoc* power analysis showed that 3D parameters for EPIC-μCT were more sensitive than 2D parameters requiring fewer animals to detect a therapeutic effect of MMPi. 2D parameters were comparable between EPIC-μCT and histopathology.
Conclusion: This study demonstrated that EPIC-μCT has high sensitivity to provide 3D structural and compositional measurements of cartilage and bone in the joint. EPIC-μCT can be used in combination with histology to provide a comprehensive analysis to screen new potential therapies.

Introduction

Degenerative joint diseases, such as osteoarthritis (OA) and cartilage injury, are a leading cause of pain, disability, and financial burden in the western world[1,2]. OA is characterized by the progressive loss of normal structure and function of articular cartilage, the smooth tissue covering the end of the moving bones[3]. This chronic disease not only affects the articular cartilage but also the subchondral bone, the synovium and the periarticular tissues[3]. People with OA and cartilage injury can experience severe pain and limited joint motion. While some promising drugs are in clinical trials, there are currently no FDA approved drugs that can modify cartilage degeneration and as such, there is a need for improved therapies and interventions.

The development of new therapeutics is a costly process involving *in vitro* screening, small animal testing, large animal testing, and eventually clinical trials. Substantial progress has been made in recent years to improve throughput, decrease costs, and provide better data with predictive indicators of the potential efficacy of a drug in humans. Much of this progress has been at the *in vitro* screening stage with techniques that include microarrays, microfluidics, and multiplex systems; however, the *in vivo* stages for screening of joint therapeutics remain slow, expensive, and with few quantitative outcomes.

In the realm of joint diseases, OA of the knee has the highest incidence[2]. In order to screen prospective therapeutics, the medial

* Address correspondence and reprint requests to: R.V. Kamath, AbbVie Bioresearch Center, 100 Research Drive, Worcester, MA 01605, USA.
E-mail addresses: nick.willett@gatech.edu (N.J. Willett), tthote@gatech.edu (T. Thote), michelle.hart@abbvie.com (M. Hart), smoran3@mail.gatech.edu (S. Moran), robert.guldberg@me.gatech.edu (R.E. Guldberg), rajesh.kamath@abbvie.com (R. Kamath).
[a] These authors contributed equally to the work in this manuscript.

http://dx.doi.org/10.1016/j.joca.2016.04.021
1063-4584/

meniscal transection (MMT) model in the rat is becoming the accepted standard as the primary *in vivo* screening model. The model displays many similar pathologic features of articular cartilage degeneration observed in human OA, including erosion and lesion formation, proteoglycan loss, subchondral bone sclerosis, and osteophyte formation[4]. This model has recently been used in numerous pharmaceutical studies to screen potential therapeutics[5–9]. Histological analysis with semi-quantitative pathological scoring is the gold standard for analysis. A major limitation of the rat MMT model is that it requires at least 3 weeks of treatment to demonstrate efficacy of potential therapeutics with a minimum of 20 animals per group[4]. The high animal requirement and long dosing period limits the testing of potential drugs in a timely and cost effective manner.

In addition to traditional histological assessment, recently developed 3D high-resolution imaging techniques provide quantitative structural and compositional analyses and have expedited the development of novel therapeutics in some fields. In the musculoskeletal field, this approach is true for μCT analysis of hard tissues, such as analyzing the efficacy of osteoporosis therapies for bone[10,11]. The ability to quantify 3D changes in bone microarchitecture provided substantial new insight into the mechanism of disease progression and accelerated the development of effective therapies for osteoporosis which are now clinically available[10,11]. Similar 3D analysis of soft tissues has made significant progress recently through techniques such as contrast enhanced imaging and 3D histology particularly for cartilage[12–16]. The contrast enhanced techniques are often based on equilibration of an ionic contrast agent into the soft tissue and then correlating the X-ray attenuation of cartilage loaded with contrast agent to structural and biological parameters[12]. Our group along with numerous other groups have developed and established a variety of a contrast enhanced techniques that allows for quantitative imaging of cartilage[13–15,17,18]. While these techniques are referred to by different names, the ionic equilibration of a contrast agent is often used and we refer to it as Equilibrium Partitioning of an Ionic Contrast Agent based micro-computed tomography (EPIC-μCT). Using this technique our group has shown that we can measure structural and compositional parameters of cartilage including proteoglycan loss, incidence of lesions and erosions, and volume of lesions; our next objective was to compare this technique to the gold standard of histopathology for the evaluation of the efficacy of a disease modifying therapy in a pre-clinical model of OA[19,20]. The application of quantitative contrast enhanced 3D imaging to cartilage has the potential to have a similar effect on drug development for OA and other degenerative joint diseases as CT imaging had on drug development for osteoporosis.

In this manuscript we utilized EPIC-μCT to evaluate cartilage degeneration during OA development and progression in the industry standard MMT model. Our objectives were two-fold: first, to perform a direct comparison of EPIC-μCT analysis to histopathological scoring looking at the development of OA in the rat MMT model; and then, to use EPIC-μCT to quantitatively assess the efficacy of a therapy for OA, in this case a broad spectrum MMPi, and similarly perform a direct comparison to the gold standard of histopathology. We hypothesized that 3D EPIC-μCT analysis would provide a more sensitive analytical method than traditional 2D techniques requiring fewer samples and allowing for the quantitative analysis of more parameters.

Materials and methods

Induction of joint degeneration

All animal studies were conducted at Abbvie Laboratories in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines and the National Institutes of Health Guide for Care and Use of Laboratory Animals. Animal facilities at Abbvie Laboratories are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Weight matched male Lewis rats (300–325 g) were subjected to MMT surgery based on previously established studies[21–23]. Two separate studies were performed. In the first study animals were randomly assigned into one of two groups: sham surgery or MMT surgery. The sham surgery was performed by exposing the joint and transecting the medial collateral ligament. In MMT animals the exposed meniscus was then transected at its narrowest point. The joint and skin were then closed with sutures. In the first set of experiments, animals were euthanized at 7 ($n = 6$ for MMT; $n = 3$ for sham), 14 ($n = 5$ for MMT; $n = 3$ for sham), or 21 days ($n = 6$ for MMT; $n = 3$ for sham). All animals had complete transections. In the second study animals were randomly assigned into one of three groups: Sham surgery ($n = 15$), MMT + MMPi ($n = 19$; two samples from the original 21 were removed due to incomplete meniscal transection identified post-mortem) and MMT + vehicle ($n = 21$). The matrix metalloproteinase inhibitor (MMPi) used in this study (biaryl ether retro-hydroxamate) is a broad-spectrum MMPi that is an AbbVie (Abbott) proprietary drug (ABT-518) and was provided by AbbVie[24]. Rats were administered the MMPi drug (30 mg/kg) in 0.02% Tween 80/2% hydroxypropylmethylcellulose (HPMC) (Sigma Aldrich, St. Louis, Missouri) orally 2×/day for 21 days post-surgery. This vehicle is used to deliver the compound (drug) via oral gavage as (1) it has no effect on disease progression in rat models, and (2) maintains the compound (drug) in suspension to enable systemic distribution. Rats in the vehicle group received 0.02% Tween 80/2% HPMC orally 2×/day for 21 days post-surgery.

Assessment of cartilage

Rats were euthanized via CO_2 inhalation at the designated time points. The tibiae and femur, including the knee joint were harvested and dissected free of surrounding tissues. The tibiae were then separated from the rest of the joint including the meniscus. The tissue was then fixed in 10% neutral buffered formalin for 3–4 days and transferred to 70% ethanol and stored at room temperature[15].

For EPIC-μCT, the proximal end of each tibia was immersed in 2 ml of 30% Hexabrix 320 contrast agent (Covidien, Hazelwood, MO) and 70% ion-free PBS at 37°C for 30 min; this is an incubation period known to result in equilibration of the agent into the cartilage and can be used to screen therapeutic efficacy[15,20,25]. Hexabrix is a non-permanent contrast agent that works based on ionic equilibration of the negatively charged Hexabrix contrast agent and correlates inversely to the amount of proteoglycan, which is also negatively charged, in the tissue. After μCT scanning all Hexabrix was washed out of the tissue as established in previous publications. Toluidine blue (used for histology, detailed below) binds based on its high affinity for acidic tissue components which is not affected by the EPIC-μCT imaging. Toluidine blue staining of tissue samples from control and MMT animals was consistent with historical observations from samples that had not undergone EPIC-μCT imaging. Proximal tibiae were blotted dry and scanned in a humid chamber using a μCT 40 (Scanco Medical, Brüttisellen, Switzerland) at 45 kVp, 177 μA, 200 ms integration time, 1024 × 1024 pixel matrix, 500 projections, 24.7 min imaging time, and a voxel size of 16 μm[25].

Following scanning, tibiae, joints, and femurs were decalcified in Cal-Ex II (Fisher Scientific, Waltham, MA) for 14 days. Dehydrated samples were routinely paraffin embedded. Coronal sections were cut at 5 μm thickness. Sections were stained with Toluidine blue.

Please cite this article in press as: Willett NJ, et al., Quantitative pre-clinical screening of therapeutics for joint diseases using contrast enhanced micro-computed tomography, Osteoarthritis and Cartilage (2016), http://dx.doi.org/10.1016/j.joca.2016.04.021