Osteoarthritis and Cartilage 22 (2014) 1886–1893

Osteoarthritis and Cartilage

Effect of muscle weakness and joint inflammation on the onset and progression of osteoarthritis in the rabbit knee

C. Egloff † ‡, A. Sawatsky †, T. Leonard †, D.A. Hart §, V. Valderrabano ‡, W. Herzog † *

† Human Performance Laboratory, Faculty of Kinesiology, University of Calgary, Calgary, Alberta, Canada
‡ Orthopaedic Department, University Hospital, University of Basel, Basel, Switzerland
§ McCaig Institute for Bone & Joint Health, University of Calgary, Calgary, Alberta, Canada

ARTICLE INFO

Article history:
Received 11 April 2014
Accepted 29 July 2014

Keywords:
Osteoarthritis
Rabbit model
Muscle weakness
Joint inflammation
BTX-A
Carrageenan

SUMMARY

Objectives: Interactions between mechanical and non-mechanical independent risk factors for the onset and progression of Osteoarthritis (OA) are poorly understood. Therefore, the goal of the present study was to investigate the *in vivo* effects of muscle weakness, joint inflammation and the combination on the onset and progression of OA in a rabbit knee joint model.

Materials and methods: Thirty 1-year-old female New Zealand White rabbits (average 5.7 kg, range 4.8–6.6 kg) were divided into four groups with one limb randomly assigned to be the experimental side: (1) surgical denervation of the vastus lateralis (VL) muscle; (2) muscle weakness induced by intramuscular injection of Botulinum toxin A (BTX-A); (3) intraarticular injection with Carrageenan to induce a transient inflammatory reaction; (4) combination of Carrageenan and BTX-A injection. After 90 days, cartilage histology of the articular surfaces were microscopically analyzed using the Osteoarthritis Research Society International (OARSI) histology scoring system.

Results: VL denervation resulted in significantly higher OARSI scores in the patellofemoral joint (group 1). BTX-A administration resulted in significant cartilage damage in all four compartments of the knee (group 2). Carrageenan did not cause significant cartilage damage. BTX-A combined with Carrageenan lead to severe cartilage damage in all four compartments.

Conclusion: Muscle weakness lead to significant OA in the rabbit knee. A transient local inflammatory stimulus did not promote cartilage degradation nor did it enhance OA progression when combined with muscle weakness. These results are surprising and add to the literature the conclusion that acute inflammation is probably not an independent risk factor for OA in this rabbit model.

© 2014 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

Introduction

Estimated costs attributable to arthritis and other rheumatic conditions (AORC) in the United states in 2003 were approximately $128 billion[1]. The Arthritis Research Center of Canada calculated the total cost of treating OA in Canada to be 1.8 billion dollars in 2010 and it is expected to quadruple over the next two decades to reach $8.1 billion by the year 2031[2]. Despite this substantial socio-economic impact, the onset, natural history, or progression of OA is still poorly understood. Scientific research over the last decade has increasingly described OA as a whole joint disease and the result of a complex interplay between several causes or risk factors.

Risk factors initiating or advancing OA can be grouped into mechanical and non-mechanical factors. Mechanical alterations, such as anterior cruciate ligament ruptures, meniscal tears, muscle weakness and malalignment, especially in joints of the lower limb, have been widely studied in animal models *in vitro*, and in human studies[3–8]. Muscle weakness and atrophy is one of the earliest signs and always accompanies OA[9]. Muscle weakness has also been shown to be an independent risk factor for OA[10] leading to increased loads in the lower limb, whereas neuromuscular training protocols seem to have a protective effect on the onset and progression of OA[11,12]. Intramuscular administration of Botulinum toxin A (BTX-A) has been used in the past to induce controlled and reversible muscle weakness and destabilization of the knee[13], and has caused weakness and significant cartilage degradation and promoted OA[8,14]. It is generally believed that abnormal mechanical

* Address correspondence and reprint requests to: W. Herzog, Human Performance Laboratory, Kinesiology, University of Calgary, Calgary, Alberta, Canada.

E-mail addresses: christian.egloff@usb.ch (C. Egloff), asawatsky@ucalgary.ca (A. Sawatsky), tleonard@ucalgary.ca (T. Leonard), hartd@ucalgary.ca (D.A. Hart), Victor.valderrabano@usb.ch (V. Valderrabano), wherzog@ucalgary.ca, ch.egloff@gmail.com, christian.egloff@usb.ch (W. Herzog).

http://dx.doi.org/10.1016/j.joca.2014.07.026
1063-4584/© 2014 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.

forces are almost always present in OA, but the question remains if they act as independent risk factors.

Non-mechanical risk factors are more difficult to define but include genetics, obesity, age, sex and inflammation. Especially Inflammation and subsequent synovitis have become an intensively studied area in the pathogenesis of OA. Although OA is defined usually as a non-inflammatory disease, there is evidence that inflammatory reactions mediated by the release of pro-inflammatory cytokines result in cartilage degradation, impaired tissue repair, and act as a trigger for symptomatic OA[15–18].

Recently, Erhart-Hledik and colleagues linked mechanical stimuli and changes in serum cartilage oligomeric matrix protein (COMP) to cartilage thinning within 5 years indicating that proinflammatory cytokines contribute to levels of soluble COMP that may be released through a trigger event, and subsequently have a detectable impact on the long term progression of OA[19]. However, to date there is no clear evidence if joint inflammation represents a primary cause for the onset of OA, or if inflammation is merely a secondary synovitis resulting from mechanical alterations that is associated, but not the direct cause of OA.

Carrageenan (a marine polysaccharide) has been used as an inflammatory stimulus in a number of experimental models (e.g., mice, rats, rabbits) for half a century. In rabbits, injection of carrageenan into the knee has been used to induce local and acute inflammation, and the temporal aspects of these inflammatory episodes have been carefully assessed using a variety of techniques[20–23].

The aim of this study was to elucidate the effects of mechanical and inflammatory interventions, and the combination of the two, on the onset and development of OA in the rabbit knee *in vivo*. Mechanical interventions included selective and general quadriceps muscle weakness, and inflammation was introduced using intra-articular injections of carrageenan. We hypothesized that muscle weakness and inflammation were independent risk factors for the development of OA, and that a combination of the two factors would accelerate osteoarthritic changes in the knee.

Methods

Experimental design

Thirty 1-year-old skeletally mature female New Zealand White rabbits (weight: average 5.7 kg, range 4.8–6.6 kg; Riemen's Furriers, St. Agatha, Ontario, Canada) were used in accordance with an experimental protocol approved by the University of Calgary Animal Care Committee. Animals were housed in pairs in floor pens to permit normal activity. Rabbits received a standard diet and water *ad libitum*. Animals were divided into four experimental groups, and one hind limb was randomly assigned for intervention (experimental hind limb) while the other hind limb served as a contralateral non-intervened control (control hind limb). For all interventions, rabbits were anesthetized using a 3.5% isoflurane (Benson Medical) to oxygen mixture.

Group 1: Animals ($n = 6$) underwent surgical transection of the nerve branch arising from the common femoral nerve leading to the vastus lateralis (VL) muscle, thereby preventing activation of VL. The VL was chosen for nerve transection because it is the biggest contributor to the extensor force in rabbits and has a laterally oriented fiber insertion into the patella, and therefore, when transected it was thought to result in considerable weakness of the knee extensor group[24], and imbalance in the knee, specifically the patellar tracking in the femoral groove.

Group 2: Animals ($n = 8$) received an injection of BTX-A (BOTOX, Allergan Inc., Toronto, Ontario, Canada) into the experimental hind limb. Injections of 3.5 U/kg of BTX-A were given once each month (day 0, 30, 60). Each injection of 3.5 U/kg was divided equally among three lines of the thigh – medial, central, and lateral – corresponding to the vastus medialis, rectus femoris, and VL muscle. The contralateral side received the same amount of sterile saline.

Group 3: Animals ($n = 8$) received an intra-articular injection of commercially available sterile Carrageenan (Sigma Chemical Co; St. Louis, MO) into the experimental knee joint using 0.5 ml of a 2% solution[21] on day 0 and day 30. This regime was chosen to make sure that the inflammatory reaction induced by the carrageenan had been resolved by the end of the study. The contralateral side received 0.5 ml of sterile saline.

Group 4: Animals ($n = 8$) received a combination of intramuscular BTX-A injection monthly and intra-articular injection of Carrageenan on days 0 and 30 of the experimental period. The contralateral side received an intra-articular and intra-muscular injection of sterile saline.

At the end of the experimental period (day 90), rabbits were sacrificed by an intravenous injection of Euthanyl (Bimeda-MTC).

Muscle mass assessment

The heads of the quadriceps muscle group (rectus femoris, vastus medialis, VL, and vastus intermedius muscle) were isolated and dissected individually and their wet mass was measured using a commercial balance with a measuring accuracy of 0.001 g (Mettler-Toledo, Switzerland).

Joint width assessment

On the day of sacrifice, the width of the experimental and contralateral knee was measured in all animals using a commercially available digital caliper. Prior to the measurement, hind limbs were shaved, the joint space between femur and tibia was identified and the largest medial-lateral diameter was marked. Measurements were repeated three times and the average value was recorded.

Cartilage histology

The femoral condyle, tibial plateau, and patella were harvested, photographed with a high resolution digital camera and fixed in a 10% neutral buffered formalin solution for 10 days (Fisher Scientific, Wohlen, Switzerland) and then decalcified with Cal-Ex II decalcifying solution (10% formic acid solution in 4% formaldehyde, Fisher Scientific) and kept at room temperature. The solution was changed daily. After 2 weeks, joints were opened and returned to a fresh decalcifying solution for an additional 2–3 weeks. Decalcification was finished when joints could be cut smoothly and without any grittiness. The joints were then washed thoroughly in running tap water for 2 h. After decalcification, the femur was cut into three pieces (femoral groove, medial and lateral femoral condyle) and the tibia into two pieces (medial and lateral plateau). Samples were then processed in an automatic paraffin processor (Leica TP 1020 Leica Microsystems, Heerbrugg, Switzerland) where they were dehydrated in a graded series of alcohols ranging between 80% and 100%, cleared in xylene and infiltrated in a mixed Paraplast-plus/extra wax (Fisher Scientific). The individual bones of the knee (tibia, femur, femoral groove, and patella) were then embedded in paraffin molds and stored at room temperature until they were sectioned. Serial sections of the knee articular cartilage were cut at 12–14 μm thickness using a Leica RM 2165 microtome. Every fifth section was collected, adhered to Superfrost Plus slides (Fisher Scientific) and allowed to dry at 40°C for 7 days. All slides were then stained with hematoxylin, Safranin-O, and fast green (Fisher Scientific). Sections were then dehydrated in ethanol, cleared in

Download English Version:

https://daneshyari.com/en/article/3379429

Download Persian Version:

https://daneshyari.com/article/3379429

Daneshyari.com