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The use of motion analysis to measure pain-related behaviour in a rat model of degenerative tendon injuries

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ABSTRACT

Chronic tendinopathy is characterized with longstanding activity-related pain with degenerative tendon injuries. An objective tool to measure painful responses in animal models is essential for the development of effective treatment for tendinopathy. Gait analysis has been developed to monitor the inflammatory pain in small animals. We reported the use of motion analysis to monitor gait changes in a rat model of degenerative tendon injury. Intratendinous injection of collagenase into the left patellar tendon of Sprague Dawley rat was used to induce degenerative tendon injury, while an equal volume of saline was injected in the control groups. Motion analyses with a high speed video camera were performed on all rats at pre-injury, 2, 4, 8, 12 or 16 weeks post injection. In the end-point study, the rats were sacrificed to obtain tendon samples for histological examination after motion analyses. In the follow-up study, repeated motion analyses were performed on another group of collagenase-treated and saline-treated rats. The results showed that rats with injured patellar tendon exhibited altered walking gait as compared to the controls. The change in double stance duration in the collagenase-treated rats was reversible by administration of buprenorphrine ($p = 0.029$), it suggested that the detected gait changes were associated with pain. Comparisons of end-point and follow-up studies revealed the confounding effects of training, which led to higher gait velocities and probably a different adaptive response to tendon pain in the trained rats. The results showed that motion analysis could be used to measure activity-related chronic tendon pain.

1. Introduction

Chronic tendinopathy refers to insidious onset of chronic activity-related pain that can affect virtually all tendons. The pathogenesis of chronic tendinopathy remains speculative (Riley, 2004). It is generally regarded as a result of failed healing to accumulated micro-injuries (Khan et al., 1999). Treatments are empirical and symptom-based, and the responses to treatment vary a lot (Alfredson, 2005). Previous experimental studies on chronic tendinopathy included analyses of clinical samples of tendinopathic tissues (Fu et al., 2002a,b, 2007; Rolf et al., 2001). Histopathological changes similar to those in clinical samples of tendinopathy were reproduced in animal models by overuse (Glazebrook et al., 2008), injection of collagenase (Chen et al., 2004) or cytokines (Stone et al., 1999). However, owing to a lack of measurement of pain, representative animal models for chronic tendinopathy could not be established. Breakthroughs for experimental studies for chronic tendinopathy will reside on the development of an objective measure of tendon pain in animal models.

A number of methods were developed to measure pain in small animals, including measurement of weight-bearing (Vrinten and Hamers, 2003), mechanical sensitivity (Fernihough et al., 2004), vocalization (Han et al., 2005) and gait analysis such as footprint analysis (Marxen et al., 2004) and motion analysis (Coulthard et al., 2002, 2003; Varejao et al., 2002). As chronic tendinopathy is characterized with activity-related pain, it is more plausible to detect the pain-associated changes in dynamic state (walking) instead of static state (standing). Gait analysis has been developed to monitor inflammatory pain in small animals such as rats (Coulthard et al., 2003), as well as large animals such as horse (Marxen et al., 2004). Coulthard et al. (2002, 2003) demonstrated the use of motion analysis as a reproducible, objective measure for acute and chronic pain induced with intraplantar injection of irritants, characterized by significant changes in temporal gait parameters such as double stance duration. Changes in ankle angle during stance phase have been used to investigate functional recovery in rats (Varejao et al., 2002). Knee joint pain induced by arthritis was widely studied in animal model by motion analysis (Neugebauer et al., 2007), but

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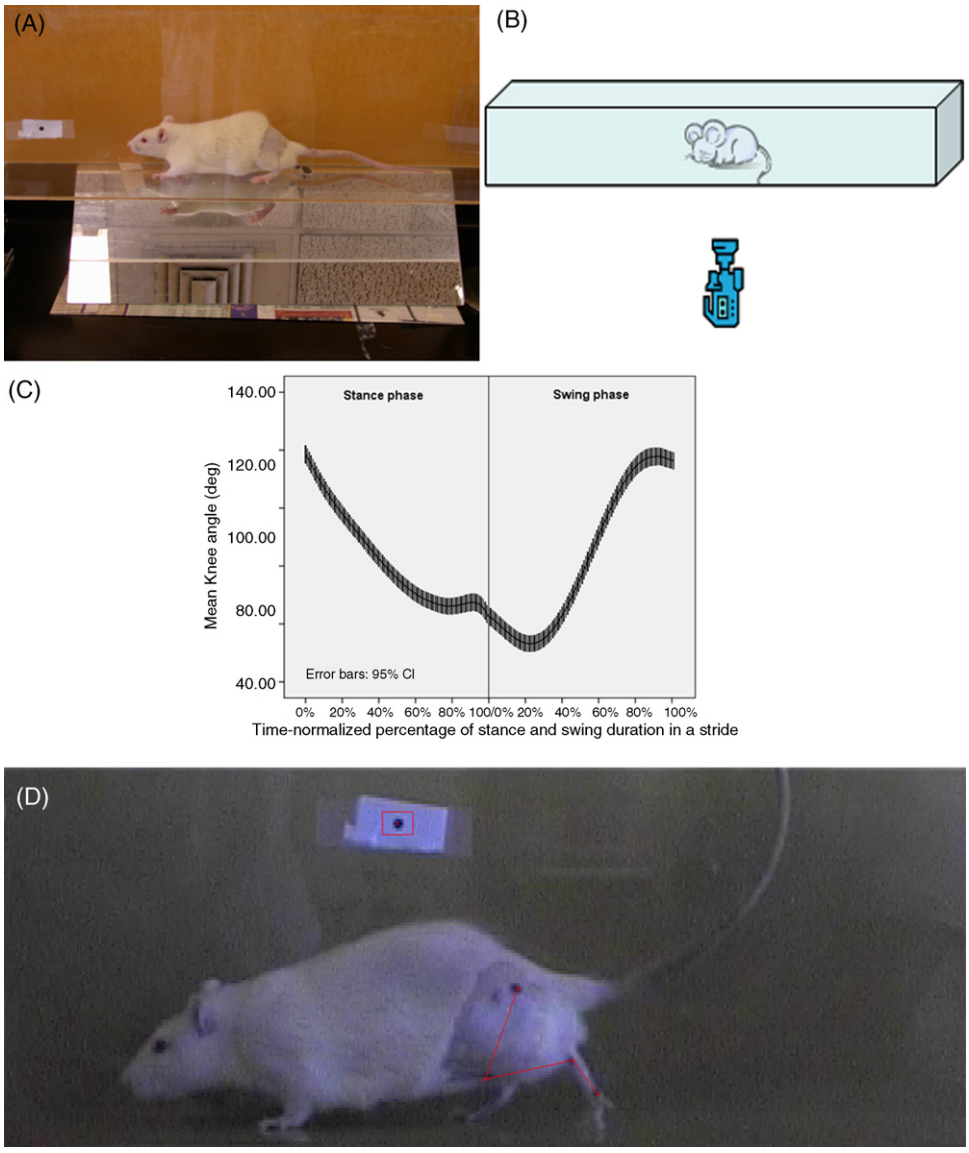

Fig. 1. Experimental set-up of motion analysis on the walking gait of rats. The rats were encouraged to walk through a transparent walkway (A) during motion analysis. The video camera was positioned to capture the rat motion in the saggital plane as shown in the diagram (B). Both hind limbs of the rats were marked on the joints of hip, knee, ankle and toe and checked for consistency of marks and joint movement by moving the limbs, as shown by a consistent kinematic plot for knee angle during a stride (C). With the help of the reference point and the marks on the joints, the gait parameters and knee angles were measured by a motion analysis system (D).

it is still unexplored if pain associated with degenerative patellar tendon injury could be monitored by the same technique.

In the current study, we reported the use of motion analysis of the saggital plane of walking gait to measure painful responses in a rat model of collagenase-induced degenerative tendon injury. Several unexplored areas in measurement of pain-associated gait changes were addressed. Firstly, changes in the contralateral side of the injured limb were analyzed in order to evaluate if there was compensatory change in the contralateral side. Secondly, two different double stance durations within a stride were separately measured. Double stance duration (DS) is defined the duration when both limbs touch the ground during a stride. It follows that two separate DS are identified in a stride: one ended with the take-off of the contralateral limb, while another ended with the take-off of the observed limb. However, there was no clear distinction between the two in previous reports (Coulthard et al., 2002, 2003). Since DS is identified as a sensitive parameter to characterize pain (Simjee et al., 2004), it is necessary to distinguish the two for the use of DS as a valid measurement for pain. Thirdly, the influences of repeated measurements as training effect were evaluated when comparing the results of follow-up study with those of end-point measurement.

2. Materials and methods

The procedures in the following animal experiments were approved by the Animal Research Ethics Committee, the Chinese University of Hong Kong.

2.1. Experimental design

Fifty-eight male Sprague Dawley rats, at an age of 8 weeks, weighting 250–300 g, were used in this study. In the end-point study, forty-two rats were randomly assigned into different groups. Six rats were subject to motion analysis without injection to the knee as pre-injection group. Thirty rats were assigned to receive collagenase injection and subject to motion analysis at 2, 4, 8, 12 and 16 weeks post injection ($n = 6$). Six rats received saline

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jneumeth.2009.02.011.

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