Background

With rates of up to 250,000 per year, anterior cruciate ligament (ACL) ruptures are one of the most common knee injuries to physically active individuals (1). Many patients who suffer ACL ruptures have persistent atrophy and weakness even after rehabilitation with deficits exceeding 30% at 6 months post-rehabilitation, when patients would typically be cleared to return to sport (2,3). Persistent weakness can lead to poor physical performance, increased risk for repeat injury as well as after knee kinematics that my promote early onset osteoarthritis (4,5,6,7). While the majority of rehabilitation interventions targeted at preventing muscle atrophy after ACL-R are focused on neuromuscular muscle weakness and fibrosis in several different models of cancer cachexia and neuromuscular diseases (11,12). Previous studies using rodent models and patients have demonstrated upregulation of myostatin after ACL tear (3,13).

Our objective was to evaluate the ability of a bioneutralizing monoclonal antibody against myostatin (10B3, GlaxoSmithKline) to prevent muscle weakness after ACL tear. Using a preclinical rat model, we tested the hypothesis that blocking myostatin activity after an ACL tear will prevent atrophy of lower limb muscles and also protect against the loss in muscle maximum isometric force production.

Methods

Animals and Surgical Procedure. This study was approved by the University of Michigan IACUC, and followed NIH guidelines for the ethical treatment of animals. The left ACL was transected in 36 Male Fischer 344 rats (Charles River, Wilmington, MA) using techniques by Delfino and Colleagues (13). Four rats served as controls. At the time of surgery, rats received a single IP injection of a bioneutralizing anti-myostatin monoclonal IgG antibody (10B3, GlaxoSmithKline) at a dose of 30 mg/kg or a sham anti-chlorohydrin monoclonal IgG antibody (E1-82.15, GlaxoSmithKline). Rats (N=8 per group) were sacrificed and tissue was harvested 7 days or 21 days after tear. Following removal of tissues, rats were euthanized by overdose of sodium pentobarbital followed by induction of bilateral pneumothorax.

EDL Contractility. Contractile properties of extensor digitorum longus (EDL) muscles were performed as previously described (14). Briefly, EDL was removed from the rat and immediately placed in a bath that contained Krebs mammalian Ringer solution supplemented with 11 mM glucose and 0.5 mM sodium pyruvate. The distal tendon of the EDL was tied to a dual-mode servomotor/force transducer (Aurora Scientific). The proximal tendon was fixed to a fixed hook. Using square wave pulses delivered from platinum electrodes connected to a stimulator (Aurora Scientific), muscles were stimulated to contract.

Histology. EDL and vastus lateralis muscles were sectioned and stained with Wheat germ agglutin (WGA) lectin conjugated to digitorum (WGA) and antibody against Myostatin (10B3, GlaxoSmithKline) using techniques by Delfino et al. (14). Quantitative PCR (qPCR) was conducted (iTaq SYBR green), and the 2^(-ΔΔCt) technique was used to normalize the expression of RNA transcripts to the stable tubocurarine chloride. The results of this study indicate that myostatin inhibition may be a promising therapeutic strategy to prevent the loss in muscle size and strength after ACL tear. While the mechanism of action is not entirely clear, based on the observed changes in expression of atrogin-1, MuRF-1, and MUSKA (which are ubiquitin ligases that are rate limiting steps in protein breakdown), and IGFea, IGFeb and 18S rRNA (which are genes that promote muscle protein synthesis), it is possible that the targeted inhibition of myostatin preserves force production by limiting the expression of proteolytic enzymes and inducing hypertrophy-related genes in the post-acute atrophy phase. Although further studies are needed, the results from this preclinical model of ACL tears suggest that therapeutic inhibition of myostatin may help prevent muscle atrophy in patients who suffer joint injuries.

Conclusions

• Persistent muscle atrophy and weakness limit the full functional recovery of patients following ACL tear.
• The results of this study indicate that myostatin inhibition may be a promising therapeutic strategy to prevent the loss in muscle size and strength after ACL tear.

References

5. Grinnell GR, Pollock L, Drucker DJ. (2003). Myostatin (GDF-8), a member of the transforming growth factor β superfamily of cytokines and functions to induce muscle fiber atrophy and weakness (8). Myostatin induces muscle atrophy by activating the ubiquitin-proteosome pathway and by blocking protein synthesis pathways activated by IGF-1 signaling (9,10). The therapeutic inhibition of myostatin has been shown to protect against atrophy, weakness and fibrosis in several different models of cancer cachexia and neuromuscular diseases (11,12).
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