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Poloxamer 188 failed to prevent exercise-induced membrane breakdown in mdx skeletal muscle fibers

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Abstract

We sought to determine the effectiveness of poloxamer 188 (P188) in protecting dystrophin-deficient, mdx skeletal muscle fiber membrane against exercise-induced breaches. mdx mice were treated with either P188 or placebo via intraperitoneal injections and run on a treadmill for 60–90 min. Membrane breakdown was quantified in cross-sections of rectus femoris muscle pretreated with Evans blue dye (in vivo). The mean % dye-penetrated muscle in the P188 and placebo groups was not significantly different in each of three trials. These results contrast with a recent report of P188 being highly effective in protecting the stretch- and dobutamine-stressed mdx heart muscle. The most likely explanations for the disparity are: (1) the exercise stress we used was beyond the protective range of P188, (2) P188 delivery and serum concentration were sub-optimal, or (3) the mdx skeletal myopathy and cardiomyopathy have fundamentally different responses to treatment.

Keywords: mdx mouse; Poloxamer 188; Sarcolemma; Dystrophin-deficient; Exercise

1. Introduction

1.1. Rationale

In mdx mice and boys with Duchenne dystrophy, the complete lack of dystrophin produces a weakened sarcolemma in all skeletal muscle fibers [1–7]. We performed these pre-clinical studies on the mdx mouse in order to determine the efficacy of a membrane stabilizer, poloxamer 188 (P188), in reducing muscle micromembrane tears induced by running exercise.

P188 is a compound that protects cells (including skeletal and cardiac muscle) from a wide range of insults to the plasmalemma [8–12]. Particularly influential for us was the work by Lee et al. [9] that showed P188 was effective in preventing skeletal muscle micromembrane tears produced by electroporation.

Treating dystrophin-deficient dystrophies with P188 is appealing on both theoretical and clinical grounds. First, the compound has the potential to protect the entire skeletal muscle fiber from the "extracellular side" of the membrane. This approach circumvents the many barriers thwarting attempts to rebuild the intracellular molecular support system. Second, P188 infusions also have the potential to treat both the skeletal myopathy and the cardiomyopathy. Recently, Yasuda et al. reported striking beneficial effects of P188 treatment of the

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mdx cardiomyopathy [13]. Third, reports of large clinical studies in young patients with sickle-cell disease and older patients with acute myocardial infarction show that short-term administration of P188 solutions is relatively safe and well tolerated [14–17].

1.2. Experimental design

We used a treadmill exercise protocol to stress the fragile mdx sarcolemma and Evans blue dye (EBD) penetration to quantify the degree of membrane disruption. We chose to study the effects of exercise on the mdx skeletal muscle because it has been shown to stress the dystrophin-deficient membrane [18–23] and contribute to dystrophic muscle damage [18,19,24,25]. Exercise stress is therefore a clinically relevant, disease-promoting factor in dystrophin-deficient muscle that, if blunted, could reduce the overall rate of muscle breakdown. Treadmill running provides excellent control of exercise with specific parameters that can be accurately measured (running time, track speed, distance run), and hence, reproduced or varied in a systematic way.

Several groups have demonstrated the value and simplicity of using EBD to expose defective membranes in mdx muscle fibers [2,7,26]. When injected intravenously or by the intraperitoneal route, EBD binds avidly to albumin and circulates freely in the extracellular compartment. The EBD/albumin complex circulates freely in the extracellular compartment. Matsuda et al. [2] first used EBD with fluorescent microscopy in mdx mouse muscle to identify fibers with incompetent plasma membranes. Fibers penetrated by EBD/albumin (those with membrane breaches) fluoresced a bright red, while fibers with intact membranes were black (Fig. 1).

In preparation for our randomized, placebo-controlled trial of P188 for the treatment of the fragile mdx sarcolemma, we established a rigorous treadmill exercise protocol, quantified the typical increase in percentage of EBD-penetrated fibers, selected the rectus femoris as the skeletal muscle for study, and defined variability of the primary measure in order to properly power our study. Because P188 can be delivered only parenterally and has a relatively short half life, the exercise period could be no longer than 90 min. In order to produce a large increased percent EBD-penetrated fibers in this limited time, we used an exhausting exercise and studied a muscle (rectus femoris) that experienced a great deal of membrane stress.

2. Materials and methods

2.1. Hypotheses and experimental design

Hypothesis 1: Exercise stress increases mdx skeletal muscle membrane breakdown when compared to non-exercised mdx skeletal muscle. Hypothesis 2: P188-treat-

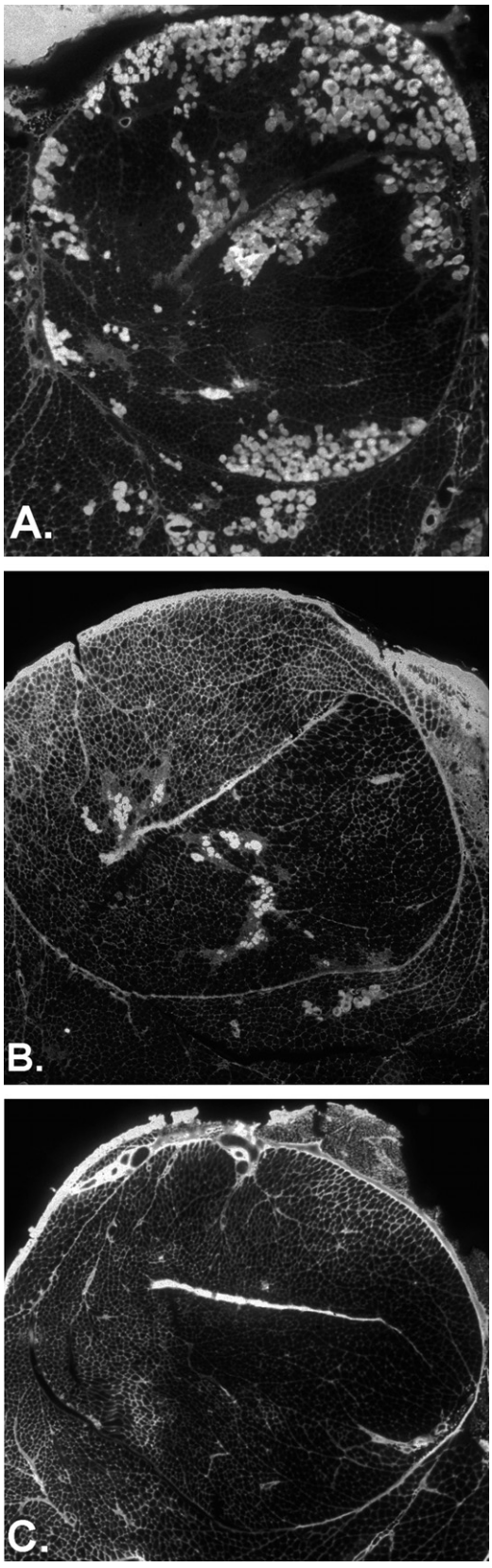

Fig. 1. Fluorescent microscopy of rectus femoris muscle after intraperitoneal injection of Evans blue dye solution: (A) mdx muscle after exhausting exercise, (B) mdx muscle not exercised and (C) wild type muscle exercised. *Note:* Black and white photographs show white dye-penetrated fibers and black unstained fibers.