COMPLEMENT INHIBITION BY C1-ESTERASE INHIBITOR IMPROVES CONTRACTILE FUNCTION IN REPERFUSED SKELETAL MUSCLE

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INTRODUCTION: Ischemia/reperfusion (I/R) injury is a pathologic phenomenon observed in microvascular surgery which results in failure of revascularized tissue. We hypothesized that activation of the classical complement pathway contributes to the development of skeletal muscle I/R injury. Human C1-esterase inhibitor (C1-INH) is a naturally occurring protein that binds to and inhibits the activated C1r and C1s. A synthetic 13 amino acid peptide derived from the sequence of C1q with a similar inhibitory action on the C1s was also examined in this study.

METHODS: Eighteen rats weighing 125-150g underwent 3 h warm innervated ischemia and 3 h reperfusion of the right extensor digitorum longus (EDL) muscle. An intravenous infusion of C1-INH (100 mg/kg), synthetic peptide inhibitor (5mg/kg, sequence: A G R P G R R G R P G L I K), or human serum albumin control (n=6 per group) lasting 10 min was started 10 min before reperfusion. After 3h reperfusion, the EDL was harvested and underwent in vitro contractile testing. The left EDL was harvested prior to ischemia or treatment and tested as an internal control. The maximal twitch force and average isometric tetanic force of the treated EDL were compared with those of the contralateral nontreated, non-ischemic EDL, and the results were expressed as a percentage of the contractile force achieved by the contralateral nontreated EDL.

After 3 h of reperfusion, blood samples were collected for determination of complement activation by measuring plasma hemolytic C3 and C4 titers. Serial dilutions of plasma samples from: 1:1,000 to 1:100,000 were incubated with C3- or C4-deficient guinea pig serum in the presence of antibody-sensitized sheep erythrocytes. Plasma C3 or C4 activities were determined by measuring absorbance at an O.D. of 412 nm to identify hemolysis and expressed as the average of O.D. 412 nm values for serial plasma dilutions.

Data analysis was performed by one-way analysis of the variance (ANOVA) and then two-way repeated-measures ANOVA to compare PBS to C1-INH and peptide-treated groups. A p-value <0.05 was considered significant.

RESULTS: Maximal twitch force increased from 35±8% (mean±SEM) of normal in controls to 48±5% of normal in C1-INH and 52±5% of normal in peptide-treated rats (Figure 1). There was no significant difference between groups. There was a significant overall increase in tetanic contractile force of the reperfused EDL in both C1-INH and peptide groups compared to control across stimulation frequencies of 70Hz, 100Hz, and 120Hz (p<0.01 by repeated measures ANOVA). Maximum improvement occurred with peptide treatment at 120Hz stimulation, with an increase in force from 38±4% in normal to 52±4% of normal in peptide-treated rats (p<0.05). There were no significant differences in contractile function between C1-INH and peptide groups.

Plasma C3 activity significantly increased in both treatment groups, from a mean O.D. 412 nm of 0.734±0.004 in controls to 0.777±0.006 in C1-INH treated rats (p<0.01) and 0.769±0.007 in peptide treated rats (p<0.01) (Figure 2). Plasma C4 activity also increased significantly in both treatment groups, from a mean O.D. 412 nm of 0.497±0.002 in controls to 0.557±0.012 in C1-INH treated rats (p<0.01) and 0.543±0.011 in peptide-treated rats (p<0.05) (Figure 3).

DISCUSSION: This study shows that treatment with either an endogenous complement C1 inhibitor (C1-INH) or a synthetic peptide inhibitor has a protective effect on skeletal muscle contractile function early reperfusion. Furthermore, both C3 and C4 activity are significantly increased in either C1-INH or peptide inhibitor-treated rats compared to control. Decreased C4 activity in the control group indicates consumption of C4, specifically by activation of the classical complement pathway; similarly, decreased C3 activity in controls is indicative of C3 consumption by activation of either the alternative or classical complement pathway. This study shows that treatment with either C1-INH or a synthetic peptide inhibitor effectively inhibits the classical complement pathway and improves skeletal muscle contractile function, suggesting that the classical complement pathway is activated in I/R injury. Inhibition of the classical complement pathway may therefore represent a potential therapeutic approach to preventing I/R injury.

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