ADDITION OF THE NO DONOR SNAC TO THE INOS INHIBITOR 1400W FURTHER IMPROVES CONTRACTILE FUNCTION IN REPERFUSED SKELETAL MUSCLE

Introduction: Nitric oxide (NO) is known to have a variety of important physiological and pathological functions. NO has been examined in various tissues after ischemia/reperfusion (I/R) injury with no clear conclusions reached. Endothelial nitric oxide synthase (eNOS) and neuronal NOS (nNOS) are constitutive forms playing important physiological roles in skeletal muscle cells. Inducible NOS (iNOS) is present in skeletal muscle at very low levels unless ischemia or inflammation occur. In these pathological processes it is dramatically induced by endotoxins and cytokines. Previous studies have suggested that a NO donor could preserve muscle contractile function following I/R injury in the initial reperfusion period. Furthermore, inhibition of iNOS has been shown to be beneficial in I/R injury by preventing inflammatory changes. The present study was designed to observe the effects of combination therapy with the NO donor S-nitroso-N-acetylpenicillamine (SNAC) and the iNOS inhibitor 1400W on contractile function of reperfused skeletal muscle. We hypothesize that the use of the NO donor in the early reperfusion period, along with the prevention of inflammation by iNOS inhibition in late reperfusion would allow improved muscle functional recovery.

Methods (approved by IACUC): 128 adult female rats weighing 180-230 grams were used. In a preliminary study to determine the ideal dosing regimen for 1400W administration, 24 rats were divided into three groups: 1) 1400W Q24 h for 24 h 2) 1400W Q12 h for 24 h 3) 1400W Q8 h for 24 h. All doses of 1400W were 3 mg/kg. 8 rats were used to study mean arterial pressure (MAP) and heart rate (HR) effects from the various drug therapies. For the main study 96 rats were randomly divided into 3 main groups based on reperfusion times of 3 hours, 24 hours, or 7 days. For each reperfusion time period, the further divided into 3 groups consisting of eight rats each: 1) Control, 2) 1400W only, 3) 1400W+SNAC, and sham surgery. All of these rats underwent 3 hours ischemia except for sham operation, followed by the specific reperfusion interval. The left extensor digitorum longus (EDL) muscle was removed from all rats and in vitro contractile testing was performed without prior ischemia. The right EDL was isolated and underwent ischemia followed by reperfusion of varying time depending on the respective group. Rats in the control group underwent left external jugular vein catheterization and received normal saline or SNAC at 100nmol/min for a total of 210 minutes, starting 30 minutes before reperfusion. Additionally, control subgroups received 0.4 ml sterile water subcutaneously 10 minutes before reperfusion, while treated groups received 3 mg/kg 1400W. For data analysis the maximal twitch force and average isometric tetanic force of the treated right EDL were compared with those of the left EDL. Two-way ANOVA with replication and one-way ANOVA were performed to compare the 4 subgroups.

Results: Ideal Dosing Regimen for 1400W Study. In the initial study to determine the ideal dosing regimen for 1400W in 24 h reperfusion rats, Q24 and Q12 hour dosing provided significant benefit compared to control while Q8 dosing did not. There was no significant difference between Q24 and Q12 dosing of 1400W. Thus for the following study one bolus dose (Q24) was used. Systemic effects of drug treatment. After administration of 1400W, MAP increased 10.5% and HR increased 15.5% over the 3 hour period. In 1400W+SNAC treated animals, MAP decreased 4% over the 3 hour period, with HR increasing 7%. For all time periods there was no significance difference from values at initiation of drug therapy. Contractile testing. EDLs of in the sham group showed significantly greater functional recovery for all tests at all 3 time periods. For 3 h reperfusion, combination therapy led to the best recovery of function of I/R groups (Fig. 1). There was statistical significance between 1400W+SNAC and control for twitch (p<0.05) and all three tetanic forces (p<.001 for 70 Hz, p<.01 for 100 and 120 Hz). Combination therapy was also significantly better than 1400W at all three tetanic frequencies (p<.05). For 24 h reperfusion, both 1400W and 1400W-SNAC showed improvement compared to control. Combination therapy allowed additional improvement vs. 1400W (p<0.05 for 120 Hz, overall tetanic function). For 7 days reperfusion, there was statistical significance between 1400W+SNAC and 1400W for overall tetanic force (p<.05).

Discussion: This study documents that treatment of reperfused rat EDL with the NO donor SNAC and the iNOS inhibitor 1400W, significantly improves return of contractile function following I/R injury. For both short-term (3 hours, 24 hours) and long-term (7 days) reperfusion periods, combination therapy was more effective than just 1400W alone. There is no functional benefit of treatment with 1400W at 3 hours reperfusion, indicating that damage resulting from overproduction of iNOS does not occur immediately after ischemia. Our findings indicate that the use of a NO donor in early reperfusion has a positive effect at all time periods, suggesting that I/R injury initially reduces levels of NO production. These results indicate that basal level of NO production in skeletal muscle is altered by the pathological process of I/R. Our study is the first to use two NO manipulating drugs together to achieve additive therapeutic benefit in contractile function. Furthermore, this is the first study of skeletal muscle I/R injury examining long-term recovery of function (7 days). We have shown that through manipulation of NO/NOS levels in skeletal muscle, significant contractile function can be preserved. These results may ultimately lead to clinical interventions for I/R injury, where combination drug therapy allows physiological and not pathological levels of NO to occur in the reperfusion period, leading to improved tissue viability.

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